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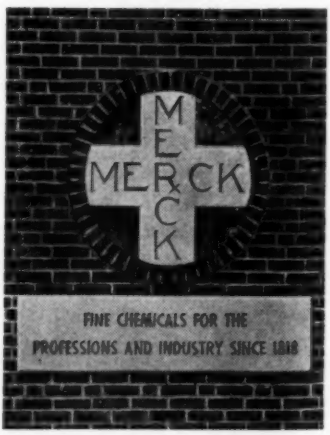

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CEREAL CHEMISTRY

VOL. XXIII MAY, 1946 No. 3

GRAIN STORAGE STUDIES. III. THE RELATION BETWEEN MOISTURE CONTENT, MOLD GROWTH, AND RESPIRATION OF SOYBEANS [1]

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(Received for publication January 30, 1946)

Moisture content is recognized as the most critical of all factors influencing the respiration, heating, and deterioration of stored grain. As the moisture content of dry grain is increased, there is a relatively slight increase in the respiratory rate until a critical moisture range, characteristic of the seed species and influenced by factors related to the commercial quality of the grain, is reached above which a rapid acceleration in respiration occurs. This has been demonstrated by Kolkwitz (1901) with barley, by Bailey and Gurjar (1918) with wheat, by Bailey (1921) with corn, by Bailey (1940) with flaxseed, rye, and other cereal grains, and by other workers with other seed types. At moisture contents favorable to rapid respiration, the heat production may be sufficient to increase the temperature of grain stored in bulk and thereby accelerate its deterioration.

Bailey and Gurjar (1918) explained the marked increase in respiration above the critical moisture range by assuming the formation of a continuous gel in the kernel structures which facilitates diffusion of the soluble nutrients to the respiratory centers of the seed located principally in the seed embryo. Bailey (1940) and Kretovitch and Uschakova (1940) admit that molds may contribute to grain respiration but only when the moisture content is well above that at which the initial rapid increase in grain respiration occurs. On the other hand, many workers including Cohn (1890), Darsie, Elliot, and Peirce (1914), McHargue (1920), Thom and LeFevre (1921), Gilman

[1] Paper No. 2274, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Degree of Doctor o, Philosophy, March, 1945.

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and Barron (1930), Swanson (1934), Larmour, Clayton, and Wrenshall (1935), Koehler (1938), Ramstad and Geddes (1942), Milner, Warshowsky, Tervet, and Geddes (1943), Leach (1944), and Milner and Geddes (1945) have stressed the importance of saprophytic mold microflora which are almost invariably present on grain, in contributing to the respiration and associated deteriorative changes. That the so-called "critical moisture content" of seeds may be associated with minimum moisture levels at which certain common molds will germinate and grow has been suggested by Gilman and Barron (1930). The work of Thomas (1937) with wheat and of Tervet (1945) with soybeans indicates that saprophytic fungi, particularly certain members of the *Aspergillus* species, may also be responsible for losses in the viability of stored grain.

The present study was undertaken to investigate the interrelationships between moisture content, mold growth, seed viability, respiration, and changes in the chemical composition of soybeans. The respiratory characteristics of soybeans of varying grade were determined at various moisture levels by a method which provided for the constant aeration of the samples at constant temperature. At the end of the respiration trials which extended over time periods up to 15 days, the seeds were examined to determine the extent of mold growth and chemical deterioration. A study also was made of the respiration of autoclave-sterilized soybeans inoculated with *Aspergillus flavus*, a fungus commonly present in commercial samples.

Materials and Methods

The soybeans used in this study included: (1) a series of naturally moist soybeans of the Illini variety grown in Illinois in 1943; (2) an exceptionally sound, well-matured sample of Wisconsin Manchu soybeans grown for seed purposes at University Farm, St. Paul, Minnesota, in 1943; and (3) a very severely frost-damaged immature sample of soybeans (sample grade, damage 55%, germination 5%) which was a composite representing the poorest quality soybeans grown in Minnesota in 1942.

Respiratory rates and respiratory quotients exhibited by the various soybean samples were determined using the apparatus and air analysis technique described by Milner and Geddes (1945), whereby the seed samples, maintained in respirometer bottles in a thermostat, were aspirated continuously with air at a relative humidity which was in hygroscopic equilibrium with the initial moisture content of the samples. Analysis of the effluent air for oxygen and carbon dioxide was performed daily.

Except in the case of the naturally moist series of soybeans, the

seed samples were conditioned to the required moisture levels by the direct addition of water, as described in a previous communication (Milner and Geddes, 1945). Moisture values were determined by the two-stage air-oven method outlined in Service and Regulatory Announcements No. 147, issued by the Agricultural Marketing Service, U.S.D.A.

Estimation of the number of seeds in a given sample infected with molds, as well as identification of the prevalent mold types, was carried out by the surface-sterilization and plating technique described by Milner, Warshowsky, Tervet, and Geddes (1943).

Oil acidity, expressed as acid value (milligrams of potassium hydroxide required to neutralize one gram of oil), was determined on the oils extracted from soybeans by the method of Zeleny and Coleman (1938). Analyses for total, reducing, and nonreducing sugars in various soybean samples subjected to respiration trials were carried out on finely ground seed samples by the methods outlined for wheat flour in *Cereal Laboratory Methods* (4th ed., 1941).

Germination values reported for certain of the soybean samples were furnished by the State Seed Testing Laboratory, University Farm, St. Paul 8, Minnesota.

Respiration of Naturally Moist Soybeans

The respiratory rates of six samples of Illini soybeans containing from 8.5% to 14.6% moisture were determined at 37.8°C over an 11-day period with samples weighing 250 g. To avoid the possibility of inhibitory carbon dioxide concentrations in the interseed atmosphere and at the same time to obtain readily measurable changes in the composition of the effluent air, two aeration rates were employed, namely, 1000 ml per day for samples containing 14.0% moisture and more, and 500 ml per day for the samples of lower moisture content.

The carbon dioxide values are given in Figure 1 in the form of the respiration-time curves for the various moisture levels. The relation between respiratory rate and moisture content on the eleventh day is also shown.

That inhibition of respiration was not involved in this trial is indicated by the data of Table I. The maximum carbon dioxide concentration in the interseed air was 1.02% which is well below the initial inhibitory level of approximately 12% previously reported by Milner and Geddes (1945).

The importance of continuous adequate aeration and of prolonging seed respiration trials for several days, stressed by Milner and Geddes (1945), is confirmed by the results of this trial. The respiration of the soybean samples containing 14% moisture and less remained low and

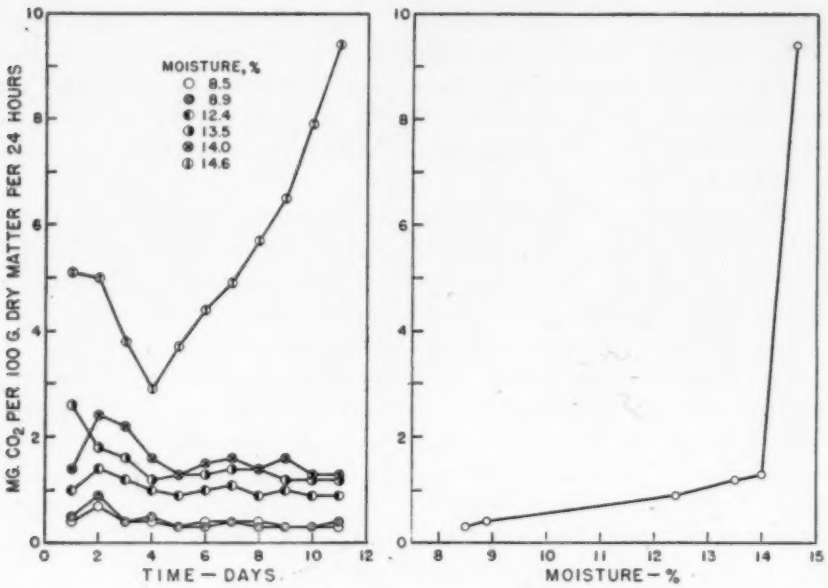

Fig. 1. Influence of time and moisture content on the respiratory rate of naturally moist Illini soybeans at 37.8°C.

relatively constant throughout the 11-day period. In contrast, after an initial decline in carbon dioxide output during the first four days, the respiratory rate of the sample at 14.6% moisture broke upwards and continued to accelerate throughout the remainder of the trial. The maximum respiratory rate of this sample was therefore not attained. The initial decline in respiratory rate shown by this sample over a period of several days may only be an apparent one since raising the temperature of the soybeans from room temperature to that of the thermostat (37.8°C) would result in the evolution of some of the pre-

TABLE I

EFFECT OF NATURAL MOISTURE CONTENT ON THE RESPIRATORY RATE OF ILLINI SOYBEANS

(Data are for the eleventh day of trial at 37.8°C with sample weights of 250 g)

Moisture	Aeration per day	CO_2 in interseed air	Respiratory rate [1]
%	*ml.*	%	
8.5	500	0.07	0.3
8.9	500	0.09	0.4
12.4	500	0.20	0.9
13.5	500	0.26	1.2
14.0	1000	0.14	1.3
14.6	1000	1.02	9.4

[1] Milligrams CO_2 per 100 g dry matter per 24 hours.

viously respired carbon dioxide retained by the seed. A similar apparent decrease in respiration was noted by Milner and Geddes (1945) in respiration trials with soybean samples freshly conditioned to various moisture levels, the decrease in initial respiratory rate being more pronounced as the moisture content of the samples increased. A similar phenomenon with soaked pea seeds has been observed by Gane (1935) who suggests that it is "probably due to an accumulation of carbon dioxide during soaking of the seed preliminary to the respiration trials."

The marked acceleration in the respiratory activity of the seeds containing 14.6% moisture, after the fourth day, as contrasted to the very low and relatively constant respiratory rates of the samples at moisture contents between 8.5% and 14.0%, suggests that the activation of some additional biological factor occurs between the narrow moisture range of 14.0 and 14.6%. Examination of the seeds at the end of the trial disclosed that only the sample at 14.6% moisture showed visible mold growth. According to the data of Ramstad and Geddes (1942), a moisture content of 14% in soybeans is in equilibrium with an atmospheric relative humidity of 74.7%, while a moisture value of 14.6% is in equilibrium with a relative humidity of 76.2%. Several investigators including Lea (1933), Galloway (1935), Barton-Wright and Tomkins (1940), and Macara (1943) have shown that over relatively short time intervals, a relative humidity of 75% is a minimum for mold spore germination at ordinary temperatures in the presence of nutrients.

The inflection of the moisture-respiration curve is much sharper than that obtained with various grains by previous investigators who usually carried out their studies over time intervals of only a few days and either aspirated off the accumulated carbon dioxide at intervals, or at the end of the trials. The present data suggest that former methods tended to underestimate the maximum respiratory potential because of the short duration of the trials, combined, in some instances, with suppression of respiration by inhibitory carbon dioxide concentrations in the interseed air, particularly at the higher moisture levels.

Respiration of Artificially Dampened Soybeans

High-Quality Soybeans. Subsamples of a sound, well-matured lot of Wisconsin Manchu seed, showing 94% germination and very low microfloral infection, were conditioned to moistures ranging from 11.8 to 18.3% and respiration trials were carried out at 37.8°C with 250 g samples aerated at 2000 ml per day, in the presence of the appropriate air humidifying solutions. The moisture contents of the samples, assayed before and after the trial, showed a maximum variation of

0.2%. The respiration data which are presented in Figure 2 emphasize the importance of the time factor in any measurement of respiratory potential. The concentrations of the carbon dioxide in the interseed air and the respiratory quotients for the two highest moistures (16.0 and 18.3%) are given in Table II for the last four days of the trial,

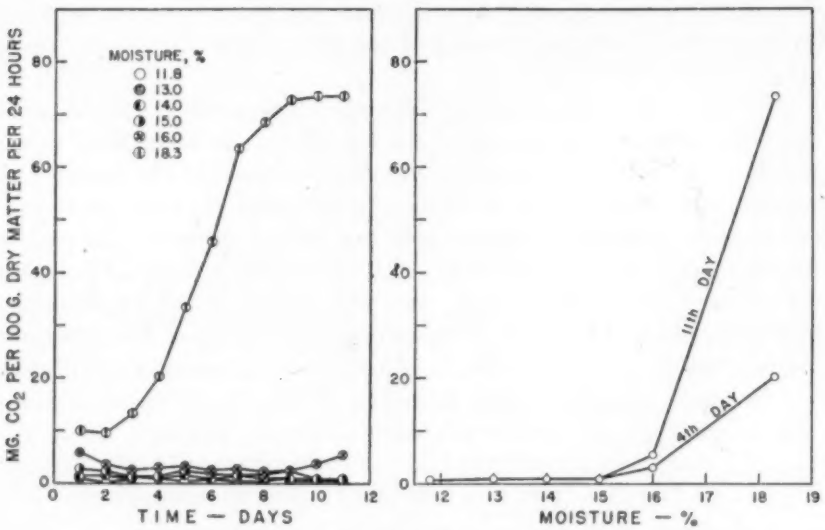

Fig. 2. Influence of time and moisture content on the respiratory rate of artificially dampened Wisconsin Manchu soybeans at 37.8°C.

TABLE II

RELATIONSHIP BETWEEN MOISTURE CONTENT, CARBON DIOXIDE CONCENTRATION IN THE INTERSEED AIR, AND RESPIRATORY QUOTIENT EXHIBITED BY HIGH-QUALITY WISCONSIN MANCHU SOYBEANS RESPIRING AT 37.8°C

Moisture		Day of trial			
		8	9	10	11
%					
16.0	CO_2 in interseed air, %	0.12	0.13	0.20	0.29
	R.Q.	0.75	0.72	0.87	0.78
18.3	CO_2 in interseed air, %	3.57	3.77	3.80	3.80
	R.Q.	0.98	0.95	0.99	0.97

Respiratory quotient values for the samples at lower moistures are not considered reliable because of the extremely small percentages of gases exchanged at the rate of aeration applied.

Initial and final moisture values, oil acidity, germination data, and observations on the extent of mold growth are reported in Table III.

TABLE III

EFFECT OF MOISTURE CONTENT ON RESPIRATION, ACID VALUE OF OIL, VIABILITY, AND MOLD GROWTH

(High-quality Wisconsin Manchu soybeans after eleven days respiration at 37.8°C)

Moisture		Respiratory rate [1]	Acid value of oil [2]	Germination	Visual condition
Initial	Final				
%	%			%	
11.8	(original sample)	—	1.0	94	Mold-free
11.8	11.9	0.5	1.0	85	Mold-free
13.0	13.2	0.9	1.1	74	Mold-free
13.9	14.0	0.9	1.3	60	Mold-free
15.1	15.0	0.9	1.5	43	Mold-free
16.0	16.0	5.4	1.8	15	Mold-free
18.3	18.3	73.3	6.9	0	Moldy

[1] Milligrams CO_2 per 100 g dry matter per 24 hours on eleventh day of trial.
[2] Milligrams of potassium hydroxide required to neutralize the acidity of 1 g of oil.

All samples at moisture values below 16.0% showed very low and relatively constant respiratory rates throughout the trial. That at 16.0% moisture gave slight respiratory increases commencing on the ninth day, whereas the one at 18.3% moisture yielded rapidly increasing respiratory activity after the second day, reaching approximate equilibrium on the ninth day. The critical moisture for this series appeared to lie between 15.0 and 16.0% (77.3% to 79.5% relative humidity) as compared to 14% (74.8% relative humidity) shown by the naturally moist series.

Visual examination of the seeds after the trial showed only the highest moisture sample (18.3%) to be moldy, although the slight increase in respiration of the sample at 16.0% moisture during the final few days of the trial indicated that mold growth was beginning. In this connection Larmour, Sallans, and Craig (1944) have pointed out that the marked increases in respiration as a result of mold growth appear well before the mold is visible to the naked eye.

Table II suggests that the respiratory quotient of soybean respiration at moisture levels favorable to mold growth increases with moisture content.

Marked increases in oil acid value of respiring soybeans did not occur until the moisture content exceeded that at which mold growth begins (Table III). Loss of viability, however, occurred throughout the entire moisture range, but was accentuated at moisture values conducive to mold growth.

Severely Damaged, Low-Quality Soybeans. Subsamples of the frost-damaged lot of soybeans were conditioned to five moisture values

in the range 10.1% to 17.0%. In view of the high degree of microfloral contamination shown by this sample which would be expected to cause high respiration values (Milner, Warshowsky, Tervet, and Geddes, 1943), seed samples of only 150 g were used and the maximum aeration rate, 2000 ml per day, was applied.

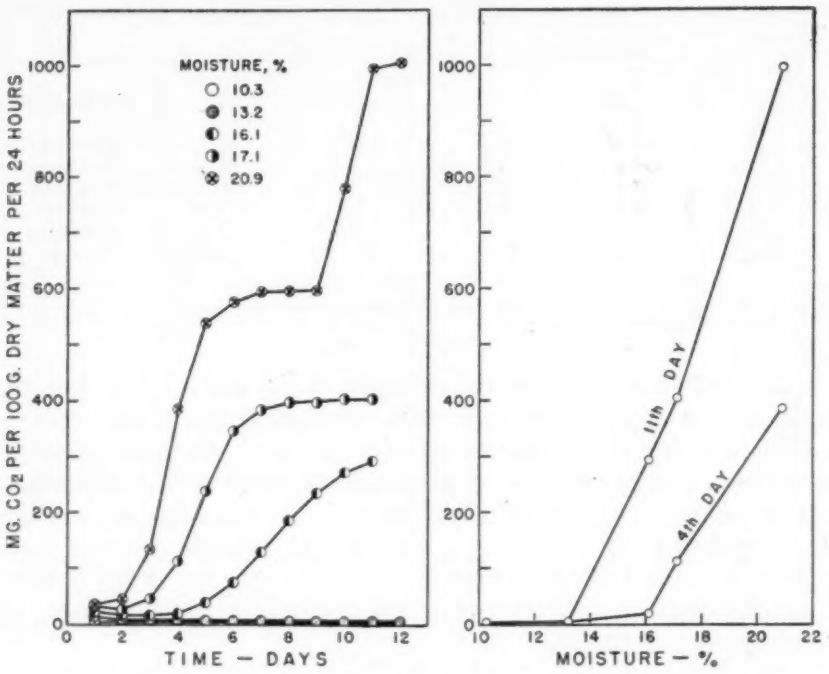

Fig. 3. Influence of time and moisture content on the respiratory rate of artificially dampened, severely frost-damaged soybeans at 37.8°C.

The data for the trial given in Figure 3 indicate that in spite of the high ratio of aeration to sample size, the respiration of the highest moisture sample was so great that by the sixth day the concentration of carbon dioxide in the interseed air reached levels inhibitory to respiration. On the ninth day, half of this sample was removed, thus effectively doubling its aeration. The relation between moisture content, concentration of carbon dioxide in the interseed air, and respiratory quotient for the last six days of the trial for the three samples of the highest moisture content is given in Table IV. Chemical, moisture, and other changes in the seeds as a result of the respiration trial are shown in Table V.

The data indicate that extremely high respiratory rates can occur in highly damaged seeds at moisture values favorable to mold growth. In this case the critical moisture value lay between 13.2% and 16.0%,

TABLE IV

RELATIONSHIP BETWEEN MOISTURE CONTENT, CARBON DIOXIDE CONCENTRATION IN THE INTERSEED AIR, AND RESPIRATORY QUOTIENT EXHIBITED BY SEVERELY FROST-DAMAGED SOYBEANS RESPIRING AT 37.8°C

Final moisture		Day of trial					
		6	7	8	9	10	11
%							
16.1	CO_2 in interseed air, %	2.38	4.15	5.98	7.52	8.68	9.36
	R.Q.	0.82	0.82	0.87	0.89	0.89	0.90
17.1	CO_2 in interseed air, %	10.94	12.15	12.65	12.61	12.73	12.71
	R.Q.	0.90	0.90	0.91	0.88	0.91	0.91
20.9	CO_2 in interseed air, %	17.39	17.90	17.97	18.05	11.80	15.03
	R.Q.	0.93	0.94	0.94	0.94	0.94	0.96

TABLE V

EFFECT OF MOISTURE CONTENT ON RESPIRATION, CHEMICAL COMPOSITION, VIABILITY, AND MOLD GROWTH

(Severely frost-damaged soybeans after 12 days respiration at 37.8°C)

Moisture		Respiratory rate [1]	Acid value of oil	Total sugars	Reducing sugars as % of total [2]	Germination	Visual condition
Initial	Final						
%	%			*mg/10 g*	%	%	
6.8	(original sample)	—	2.9	—	—	5	Mold-free
10.2	10.3	0.6	3.8	290	44.1	6	Mold-free
13.1	13.2	3.3	4.2	291	43.6	0	Mold-free
15.1	16.1	291.7	14.5	268	51.1	0	Some mold
16.1	17.1	400.9	22.7	239	57.3	0	Moldy
17.0	20.9	1003.8	56.4	219	70.3	0	Very moldy

[1] Milligrams CO_2 per 100 g dry matter per 24 hours on twelfth day of trial.
[2] Reducing sugars are calculated as maltose; nonreducing as sucrose.

apparently well below the 16% value for the high-quality seeds (Figure 2). These results appear to be in line with the findings of Tomkins (1929) since they demonstrate that increased nutrient availability to the molds, as might be expected with frost-damaged seeds, tends to lower the humidity requirements for mold spore germination. Such an increase in the readily available nutrients present on the damaged seeds would result also in a greater extent of mold proliferation, as evidenced by the very high respiratory rates reached by the damaged sample. Thus sound seeds such as those of the high-quality sample, with their smooth unbroken seed coats, present a more inhospitable medium for mold mycelial penetration and growth than do the highly frost-damaged seeds, in which the majority of the seed coats were ruptured,

and a large part of the sample consisted of naked cotyledons entirely devoid of seed coats. At a moisture content of 18.3% the high-quality sample showed a maximum respiratory rate of 73.3 mg carbon dioxide per 100 g dry weight per 24 hours; while by interpolation, the frost-damaged sample has a rate of about 600 mg at this moisture level. These values indicate that a much greater hazard is involved in the storage of damaged as compared with sound damp soybeans.

The tendency of the respiratory quotient to increase with moisture contents corresponding to relative humidities favorable to mold growth, as noted in the previous experiment, is also shown by the data in Table IV. Milner and Geddes (1945) observed a similar trend with samples of approximately equal moisture content (18.5 to 19.3%) when the aeration rates were increased from low values at which respiratory inhibition occurred.

The very marked increases in the respiratory rate of damaged seeds with time, at moisture values permitting mold growth, are reflected in profound changes in chemical composition (Table V). The greatest relative change occurred in the degree of fat hydrolysis, as indicated by the acid values of the extracted oils. Total sugars decreased while reducing sugars underwent a marked increase.

A striking result of this experiment, in which respiratory rates reached very high rates, was the marked increase in the moisture content of samples on which mold growth occurred.

The Respiratory Behavior of Sterile Soybeans Inoculated with Aspergillus flavus

Mycological examination of the high-quality Wisconsin Manchu seeds at the end of the respiration trials showed that the sample at 18.3% moisture was contaminated with *Aspergillus flavus* and *A. glaucus*. *A. flavus* has been shown by Thomas (1937) and Tervet (1945) to exert a toxic effect on seed viability. These observations prompted a study of the respiratory characteristics of this mold by inoculating sterile soybeans with spores grown in pure culture. By this means any biological activity due to the seeds themselves was eliminated.

Six lots of the high-quality samples were conditioned to various moisture contents in stoppered Erlenmeyer flasks for four days at room temperature, with occasional shaking. With cotton plugs replacing the stoppers, the samples were autoclaved for one hour at 120°C. A slight browning of the seeds resulted from this treatment. In the same autoclave were placed a number of eight-ounce bottles to be used as respirometers, which were previously tared with the stoppers and connections used for assembling the respirometers. All openings to the

respirometers were previously plugged with glass wool. Observing sterile precautions, the sterile cool seeds were inoculated with a large number of spores of *Aspergillus flavus* from a week-old pure culture grown on an agar slant. Five samples of varying moisture content were inoculated with mold spores, while a sixth, at the highest moisture, was left sterile to serve as a control during the respiration trials. Portions of the inoculated samples were transferred to the tared respirometer bottles which were closed with the sterile stoppers through which passed the glass wool plugged connections. Seed sample weights in the respirometer bottles were determined by weighing the sealed bottles and their contents. The samples ranged in weight from 109 to 146 g.

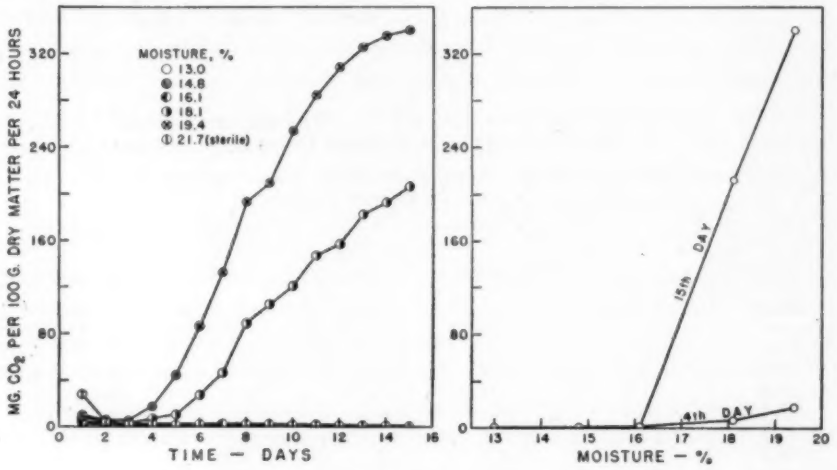

Fig. 4. Influence of time and moisture content on the respiratory rate of sterile soybeans inoculated with *Aspergillus flavus* at 37.8°C.

Respiration trials were conducted at 37.8°C and an aeration rate of 2000 ml per day, with results shown in Figure 4. The relationship between moisture content, carbon dioxide concentration in the interseed atmosphere, and respiratory quotient for the inoculated samples at the three highest moisture levels during the last four days of the trial is shown in Table VI. Changes in the chemical composition and the visual condition of the seeds are recorded in Table VII. The initial chemical values are for determinations made on the samples after autoclaving but before the respiration trial; any effects of autoclaving on chemical composition are not involved.

The close similarity of the respiratory characteristics of a pure mold culture growing on a sterile soybean substrate to those of normal seeds at similar moisture values indicates that the greatest part of the

TABLE VI

RELATIONSHIP BETWEEN MOISTURE CONTENT, CARBON DIOXIDE CONCENTRATION IN THE INTERSEED AIR, AND RESPIRATORY QUOTIENT EXHIBITED BY AUTOCLAVE-STERILIZED SOYBEANS INOCULATED WITH *Aspergillus flavus*, AT 37.8°C

Final moisture		Day of trial			
		12	13	14	15
%					
16.1	CO_2 in interseed air, %	0.00	0.00	0.00	0.00
	R.Q.	—	—	—	—
18.1	CO_2 in interseed air, %	3.58	3.93	4.38	4.83
	R.Q.	0.98	0.97	1.01	1.00
19.4	CO_2 in interseed air, %	6.96	7.35	7.58	7.69
	R.Q.	0.99	0.98	0.99	0.99

TABLE VII

EFFECT OF MOISTURE CONTENT ON RESPIRATION, ACID VALUE OF OIL, AND MOLD GROWTH

(Autoclave-sterilized Wisconsin Manchu soybeans inoculated with *Aspergillus flavus*, after 15 days respiration at 37.8°C)

Moisture		Respiratory rate [1]	Acid value of oil		Visual condition
Initial	Final		Initial	Final	
%	%				
12.9	13.0	0.0	1.3	1.0	Mold-free
14.8	14.8	0.0	1.3	1.0	Mold-free
16.4	16.1	0.0	1.3	1.0	Mold-free
17.4	18.1	212.0	1.4	1.9	Moldy
18.9	19.4	340.2	1.3	3.8	Moldy
21.9	21.7	0.3	1.5	1.1	Mold-free
(sterile control)					

[1] Milligrams CO_2 per 100 g dry matter per 24 hours, on fifteenth day of trial.

sharp increase in respiration of normal wetted seeds can be attributed to mold proliferation commencing at a sharply defined minimum humidity. The small but measurable amount of respiration noted with normal seeds at low moisture values is absent in the sterile mold-inoculated seeds in a similar moisture range and suggests that this very slight carbon dioxide evolution is due to seed respiration.

The critical point in the moisture-respiration curve occurred slightly above 15% for the normal, sound sample (Figure 2), and above 16.1% moisture for the sterile, inoculated sample. The normal seed was contaminated with both *Aspergillus flavus* and *A. glaucus* and the latter fungus proliferated more freely during respiration trials. The lower critical moisture value for the normal seed suggests that *A.*

glaucus has a lower humidity requirement than *A. flavus*. This is confirmed by the data of Koehler (1938) who showed that the minimum moisture requirement for the growth of *A. glaucus* on shelled corn was 14%, while that for *A. flavus* was 18%. Similarly, Galloway (1935) found that spores of *A. glaucus* would germinate in the presence of nutrients at relative humidities as low as 75%, while spores of *A. flavus* required 85% relative humidity. These humidity values correspond to equilibrium moisture levels of 14.1% and 18.0% in soybeans, according to the data of Ramstad and Geddes (1942).

The values for the initial and final moistures of the samples in this trial confirm the previous observation that mold growth and respiration cause moisture accumulation in the seeds. The inoculated samples which underwent no respiratory increases, as well as the high moisture control sample, showed no significant moisture changes.

Table VI indicates that no significant difference exists in the respiratory quotient of a mold when respiring at different moisture levels on a biologically inert substrate. This is in contrast to the respiratory characteristics of the same sample of normal seed where both seed and mold respiration are involved. The data show conclusively that the respiratory quotient of purely mold respiration is 1.0, under the conditions of this experiment, with soybeans. These facts lend confirmation to the hypothesis previously suggested that the respiration of the soybean seed itself is not only of a very low order of magnitude, but is also characterized by respiratory quotients well below unity. That this is the case was shown by a study of the respiratory behavior of a series of samples of the sound Wisconsin Manchu soybeans at moisture values below the critical. These data, presented in Table VIII, were

TABLE VIII

EFFECT OF MOISTURE CONTENT BELOW THE CRITICAL LEVEL ON RESPIRATORY RATE AND RESPIRATORY QUOTIENT

(High-quality Wisconsin Manchu soybeans on tenth day of trial at 30°C)

Moisture	Respiratory rate [1]	R.Q.
%		
9.3	0.11	0.50
11.3	0.21	0.56
12.4	0.34	0.67
13.4	0.41	0.73
14.3	0.56	0.76

[1] Milligrams CO_2 per 100 g dry matter per 24 hours.

obtained by using very large seed samples and low aeration rates, in order to obtain readily measurable changes in the composition of the respired air. It may be concluded that where mold growth on seeds

is restricted by lack of air, high carbon dioxide concentration, or unfavorable moisture, the combined respiration of the seed and mold would yield a respiratory quotient intermediate between the low value due to the soybean seed and the value of 1.0 for purely mold respiration. Conversely, as mold respiration increases with increasing aeration and moisture, the predominant respiration of the molds forces the average respiratory quotient very close to unity.

Discussion

Two agencies are responsible for the respiration and deterioration of soybeans under practical storage conditions where insect life is not involved, namely the normal metabolic processes of the seed itself and the biological activity of molds, which are almost invariably present.

At moisture values below 14%, seed respiration only is involved, and changes in chemical composition and viability proceed at a very slow rate. The energy released by seed respiration is apparently too slight to promote the heating of seeds when stored in bulk. However, even in this moisture range, there is a loss in viability with increasing moisture, particularly when the seeds are stored at a relatively high temperature. The present studies confirm similar observations reported by Barton (1941) and Ramstad and Geddes (1942).

The slow decrease in germination which occurs on the storage of "dry" seeds at ordinary temperatures may possibly be associated with the extent of respiration, slight as it is under these conditions. The production of carbon dioxide is enhanced by increased moisture and temperature, and unless sufficient moisture is provided for germination, the accumulating carbon dioxide or other respiration products may reach levels toxic to the embryo. In this connection, Kidd (1916) has suggested that carbon dioxide acts as a narcotic to seeds thereby inducing dormancy, and that germination is related to a lowering of this inhibitory partial pressure of carbon dioxide in the tissues.

The present studies have yielded data which show that normal viable soybeans release carbon dioxide when wetted, or when their temperature is raised, over a period of a few days, before either normal seed or mold respiration becomes established. That this carbon dioxide release is probably not due to a high initial rate of respiration seems to be indicated by the fact that high respiratory quotients in the range of 1.5 to 2.0 appear during this process and fall to normal values when this "bound" carbon dioxide is dissipated. This observation is not in accord with the claim of Freitinger (1927) that dormant seeds have a continuing intramolecular type of respiration, owing to the low oxygen permeability of intact seed coats, until sufficient moisture is provided to swell and rupture the seed coat. Furthermore, Milner

and Geddes (1945) demonstrated that very high sustained respiratory quotients indicative of anerobic respiration occurred in damp soybeans only when the seeds were maintained under nitrogen (containing 0.2% oxygen).

In the present studies, normal dormant soybeans respiring in air over extended time intervals exhibited an increasing trend of respiratory quotient between 0.50 and 0.76 in a moisture range between 9.3 and 14.3% (Table VIII). Meyer and Rader (1936) have shown that germinating soybeans, which doubtless respire at a considerably higher rate than "dry" dormant soybeans, exhibit a respiratory quotient of 0.65. Under the same conditions, the respiratory quotient of germinating wheat was 1.0. These observations suggest that dormant soybean respiration is of a normal aerobic type, but that substrate materials other than carbohydrates, possibly fats, are respired by soybeans. Another interpretation of the regular increase in respiratory quotient as the moisture approaches that favoring mold growth is a binding of respired carbon dioxide by the tissues in the face of otherwise normal aerobic respiration, the degree of retention of carbon dioxide decreasing with increasing moisture.

When the moisture content exceeds a minimum value (usually about 14% but dependent on a number of factors), a very sharp increase in the respiratory rate of soybeans occurs; this indicates that an additional and very active agency contributing to the respiration has come into play. The evidence is quite conclusive that molds are responsible. The present studies have shown that their respiratory quotients are very close to a value of 1.0, indicating that as far as the principal species encountered in these studies are concerned (*Aspergillus flavus* and *A. glaucus*), aerobic carbohydrate utilization is the major process.

The minimum moisture content of soybeans where the sharp upturn in respiration has been found to occur, namely 14%, corresponds to an equilibrium relative humidity in the surrounding atmosphere of approximately 75%. This humidity level is the minimum critical humidity at which the most xerophytic mold species proliferate. Walter (1931) has stressed the fact that various microfloral species have different minimal moisture requirements for growth, and Galloway (1935) has classified a large number of common molds of the genus *Aspergillus* and *Penicillium* on the basis of their minimal humidity requirements for germination and growth on nutrient media. In general, the *Aspergilli* include the most xerophytic of all mold species. Most of the members of this group which have been studied initiate growth in the presence of nutrients in the range of humidities from 75% to 85% in time intervals up to two weeks. The *Penicillia* appear to

require a minimum humidity of 85%, while other species including *Rhizopus*, *Trichoderma*, and *Cladosporium* require minimum relative humidities of about 90% for spore germination. Bacterial spore germination and growth is not likely to be involved under normal conditions at relative humidities below 95%. In the present studies it was noted that the proliferation of *Aspergillus glaucus* on soybean seeds occurred at a moisture level some 3% lower than was required for the growth of *A. flavus*.

That the relative humidity of the atmosphere surrounding natural products rather than the hygroscopic moisture values appears to govern mold spore germination and growth is clearly indicated when the data of several investigators are correlated. Haines (1937) emphasizes this point in a review on the causes of the microbiological deterioration of meat. Lea (1933) found that lipolysis due to mold growth commenced on bacon fat at 75% relative humidity. Macara (1943), after investigation of the causes of mold growth on dried meat, found that on meat containing 40% fat and 10% water, the moisture noted being in equilibrium with a relative humidity of 74–75%, molds might grow after a few weeks. Rozsa (1935) in a review of the work of Pap (unpublished) concluded that a relative humidity of 75% initiated mold growth with resulting increases in oil acidity in stored wheat. Smith (1938) has provided an excellent analogy to explain these facts with the statement: "If dry samples of pure wool and pure cotton are exposed to the same (humid) atmosphere, the wool will take up approximately twice as much moisture as the cotton, and, leaving out differences due to chemical composition, the two samples will be approximately equally liable to mildew. . . ." Since the completion of the present studies, Snow, Crichton, and Wright (1944) have demonstrated that extremely wide variations in equilibrium hygroscopic moisture exist in different natural feedstuffs at common relative humidities. Their data confirm the conclusion that mold growth is determined by the humidity factor rather than the actual moisture content of the material.

A conclusion of fundamental importance is that on as diversified a range of natural products as wheat, bacon fat, meats, leather, textiles, flour, feeding stuffs, and book-binding materials, the minimal relative humidity at which certain mold spores, commonly contaminating such materials, may grow, is virtually the same. In other words, a narrow humidity range with a minimum of about 75% corresponds to the different "critical" moisture levels shown by a variety of natural products, and it is this humidity which is associated with the initiation of mold growth on all. Rozsa (1935) expresses this thought as follows: "The source (of moisture for the growth of microorganisms on wheat)

is mostly the vapor content of the atmosphere of the storeroom, or the air layers wrapping wet kernels of wheat which are so rich in vapor. Not the actual moisture is responsible for the damage of the different mill products, as the danger occurs at very different moisture contents. The damage begins at identical relative vapor pressures—otherwise at the biological equivalent of the moisture content."

The critical humidity level (75%) at which mold growth is initiated is probably in hygroscopic equilibrium with the different moisture levels in various seed species (e.g., flaxseed 10.5%, wheat 14.5%) where upward respiratory trends, similar to those shown in the present studies with soybeans, have been reported. The data presented have shown that if inhibitory interseed carbon dioxide concentrations are avoided, the well-defined upward trend in respiratory rate at the critical humidity for mold growth occurs more sharply than previous reports have suggested. In order to determine the "safe" moisture limits for a grain whose storage characteristics are unknown, it had been the practice in the past to determine its respiration rate at various moisture levels and then to designate as "critical" that moisture value at which the sharp upward trend in respiration commences. That this laborious and time-consuming procedure is unnecessary is evident from the discussion given on the relationship of relative humidity to mold growth. With certain reservations to take into account factors due to seed condition, one need only determine at ordinary temperatures, the relative humidity-hygroscopicity moisture relationships of any material in order to designate as "critical" that moisture value which is in equilibrium with a relative humidity of 75%.

Bailey (1940) questioned the suggestion of Rozsa (1935) that mold growth can occur at relative humidities as low as 75%, and pointed out that even if this were possible, the equilibrium moisture content of wheat at this humidity, according to the data of Coleman and Fellows (1925), is 17 to 17.5%, which is well above the critical moisture in the range of 14 to 15% where the sharp upward break in the respiratory activity of wheat was shown to occur by Bailey and Gurjar (1918). On this basis, Bailey concluded that molds would not proliferate until the moisture content of wheat was in excess of 17.5%, and that phenomena other than mold growth are responsible for the rapid increase in wheat respiration in the critical moisture range. Bailey (1940) failed to note that the moisture data of Coleman and Fellows (1925) are expressed on the dry weight basis, and not on the conventional "as is" basis. When recalculated, their values show that 75% relative humidity is in equilibrium with a moisture content of 14.6% and not 17.5%. Their results then fall into close agreement with the hygroscopic moisture data for wheat published by Rozsa (1935). This,

figure, 14.6%, incidentally, coincides with the minimum of the moisture range which Bailey and Gurjar (1918) termed critical for wheat, and, as has been pointed out, also agrees with published data on the minimum relative humidities at which certain members of the *Aspergillus* species of molds will germinate and grow.

The critical humidity at which mold spores will germinate and yield mycelial growth is affected by various factors the most important of which are time, temperature, and nutrient availability. Thus, McHargue (1920) noted that molds did not appear on whole corn until a moisture of 15% was reached, whereas mold growth developed on corn meal at moistures as low as 12%. Wilson (1928) has pointed out that mold growth on soybeans appears first in the cracks and broken places of the seed coats. Tomkins (1929) found that the latent period of germination of mold spores decreases with increasing availability of nutrients. Increased nutrients also increase the range of humidity and temperature over which spores will germinate and grow. Tomkins concluded that "when any one of the conditions is modified so as to favor growth, that is, make it more rapid, the limitation of the growth rate by variation of any other condition is rendered more difficult." Similar conclusions were reached by Groom and Panisset (1933) after a thorough study of the causes of mildewing of book materials. Barton-Wright and Tomkins (1940) found essentially the same relationships to hold true for the growth of molds in wheat flour. The critical importance of the time factor has been stressed by Snow, Crichton, and Wright (1944) who point out that while 75% relative humidity may be considered as a safe humidity for storage of most feedstuffs for time intervals up to three months, they were nevertheless able to detect spore germination and some mycelial growth in feeds after three years of storage at humidities as low as 65%.

The present studies have shown that the latent period of mold spore germination, corresponding to the time interval preceding rapid respiratory increases in damp soybeans, decreases with increasing moisture content. Furthermore, frost-damaged seeds show considerably shorter lag periods before the occurrence of respiratory increases due to mold growth, as well as much higher maximum respiratory potentials than did sound, high-quality seeds at comparable moisture levels. Frost-damaged seeds also showed somewhat lower critical moisture levels. These differences can be ascribed directly to the greater concentration and availability of mold nutrients in the damaged seeds, as well as to a greater degree of initial mold contamination. In this connection, Milner, Warshowsky, Tervet, and Geddes (1943) have shown that frost damage in soybeans is accompanied by increases in phosphate acidity, nonprotein nitrogen, and reducing sugars. In general, it

would appear that the greater storage hazards encountered with immature grain, or grain damaged by mechanical injury, by freezing, sprouting, or weathering, as compared to well-matured grain at similar moisture values, can be ascribed to the fact that such grain provides a more favorable medium for mold growth than does normally matured, carefully harvested grain.

An interesting conclusion to be drawn from the data reported in the present studies, as well as that from the literature which has been reviewed, is that, of the large variety of microfloral species which usually contaminate normal soybeans, only a small group of common molds (certain species of *Aspergillus* of which *A. glaucus* is of greatest importance, and a very few *Penicillia*) are involved in the respiration and storage deterioration of soybean seeds within the moisture ranges normally encountered. It is notable that certain members of the *Aspergilli*, particularly *A. flavus*, which have been considered to be saprophytes, preferring dead organic media for growth, can grow on normal seeds and adversely affect their germination. That the metabolic activity of molds on stored grain may lead to marked increases in the moisture content of the seeds is clear from the data of this study. This is indicative that the mycelial mat which appears on moldy seeds is considerably more hygroscopic than are the seeds themselves, at the same relative humidity.

Summary

The influence of moisture content on the respiratory characteristics of soybeans of varying commercial quality at 37.8°C was studied by a technique providing for the simultaneous measurement of oxygen consumption and carbon dioxide production, under conditions of continuous and controlled aeration, for time intervals up to 15 days.

Moisture values below about 14% yielded very low and virtually constant respiratory rates over extended time intervals, indicative of purely seed respiration. Small increments of moisture beyond this value were accompanied by respiratory increases over a period of several days until equilibrium respiratory conditions were approached. This effect was due to mold growth, and the plotted respiration values thus assumed the form of a microbiological growth curve. Accordingly with time, before equilibrium values were reached, increasingly sharper inflection of the respiration curve in the critical moisture range was noted.

Latent period of mold spore germination, corresponding to time intervals preceding rapid respiratory increases, decreased with increasing moisture content. Frost-damaged seeds showed shorter respiratory lag periods and considerably higher respiration rates than did

high-quality soybeans at similar moisture levels, as well as a significantly lower critical moisture value than sound, well-matured seeds. These differences are ascribed primarily to the greater concentration and ease of availability of nutrients for mold growth in damaged as compared with sound seeds.

Respiratory characteristics of autoclave-sterilized (biologically inert) soybeans at various moisture levels, inoculated with spores of a mold indigenous to normal soybeans, showed the same characteristic sharp upward trend in respiration at a critical moisture value as did normal seeds; the low respiration values yielded by normal seeds at lower moisture values (seed respiration) were absent.

Wetting or heating of normal soybeans caused an initial evolution of carbon dioxide apparently not related to respiration, which is attributed to the release of preformed carbon dioxide bound by the dormant seeds.

Respiratory quotients increased with moisture content and approached 1.0 as the ratio of mold to seed respiration increased. When seed respiration was absent (autoclaved, mold-inoculated seeds), the respiratory quotient of 1.0 exhibited by the mold respiration was unaffected by moisture content.

Viability of soybeans decreased somewhat when the seed was held at 37.8°C at moisture contents below that favoring mold growth, but was most adversely affected at moisture contents where molds grew readily.

Only slight changes with time in the chemical composition of respiring soybeans as estimated by oil acid value and total and reducing sugars were noted at moisture values unfavorable to mold growth. At moisture levels where molds proliferated, drastic chemical changes occurred, approximately proportional to the moisture content and the extent of mold growth.

Significant increases in the moisture content of soybeans maintained in atmospheres of constant humidity in equilibrium with the seed moisture were noted when mold growth was extensive. At the same relative humidity, mold mycelia are probably more hygroscopic than are soybeans.

Aspergillus glaucus and *A. flavus* were the principal microflora which proliferated on soybeans in the course of respiration trials, in the moisture range studied. *A. glaucus* was the most xerophytic species encountered, appearing to initiate growth at about 14% moisture in soybeans (in time intervals within two weeks) corresponding to a relative humidity of 75% in the surrounding atmosphere. *A. flavus* required a moisture value some 3% higher for germination.

The literature reviewed indicates that only a few mold types are

capable of germination and growth at relative humidities as low as 75%, whereas an increasing diversity of microfloral species appears at higher humidities.

Relative humidity rather than actual moisture content of seeds determines their susceptibility to molding. It is concluded that the various "critical" moisture values for different seed species (e.g., flaxseed 10.5%, wheat 14.5%) are those moisture contents which are in hygroscopic equilibrium with a common relative humidity of about 75% which, over moderate time intervals, is the minimal humidity required for the growth of the most xerophytic mold species which contaminates the seed.

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A STUDY OF THE MILL STREAMS COMPOSING 80% EXTRACTION FLOUR WITH PARTICULAR REFERENCE TO THEIR NUTRIENT CONTENT

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The composition in terms of the B vitamins, thiamine, riboflavin, and nicotinic acid of individual mill streams in white flour milling has been studied by Jackson, Doherty, and Malone (1943). The particular interest of the present study is that it represents a survey of the streams of a commercial mill making 80% National flour of relatively high vitamin content despite relatively short surface.[1] Thus it covers the adjustments and devices aiming at securing a substantial entry of the germ, particularly the scutellum, into the flour as a whole, which are now typical of British practice. These features were discussed by Kent, Simpson, Jones, and Moran (1944); also they are well summarized by J. F. Lockwood (1945). They will be described briefly in the present paper.

Milling Procedure

The tests were made with the cooperation of the Coxes Lock Milling Company Ltd., Weybridge, Surrey, a country mill with a capacity of 20 sacks (of 280 lb.) of flour per hour, i.e., in American terms, 686 barrels per 24 hours.

At the time of the test the grist was composed of 60% Nos. 1 and 2 Manitoba and 40% English wheat. The extraction actually obtained was 80.5% of National flour. This was calculated on total products with screenings and wheat cleanings running automatically through a grinder into the offal. The straight run or National flour was better than average in color, its color score (Moran, 1944) being about 10 as against a national average of 15, with a thiamine value above average (0.89 as against a national average of 0.80), and was of good baking quality.

The mill has four breaks (the fifth, put into operation when milling 85% flour, was abandoned on changing to 80% extraction), but the last three have coarse and fine subdivisions. The total break roll surface was only 26 inches per sack of 280 lbs. flour per hour, the total reduction roll surface 48, and *X* and *Y* 6, the total being 80, or only 70% of that regarded as typical of British white flour milling.

[1] In the United Kingdom, percentage extraction is calculated on total products including screenings. It is, therefore, about 2% higher than if calculated on clean dry wheat.

British and American Milling. In the following account wherever possible the equivalent American milling terms have been inserted in brackets after the corresponding English terms. It should be noted, however, that there is no general American or Canadian equivalent to the English scratch system—including *X* and *Y* rolls. This may be briefly explained in American terms as follows: The throughs ("brown sizings") of the last sheets of the first and second purifiers are sent to *X* roll which is fluted with 28 corrugations per inch and driven at $2\frac{1}{2}:1$ differential. The tails ("chunks") of these purifiers, and also of the third purifier, are sent to a similar roll called *Y*. *Y* also receives the coarsest and most branny parts of the grind from *X*—as separated by *X* scalper and by *X* middlings purifier; the other parts pass to the early middlings reductions. The coarser part of *Y* grind is scalped to offal but its middlings are purified (*Y* purifier) and contribute respectively to the various coarse or tailings rolls. Thus instead of chunks being sent *direct* to tailings rolls (or IV *Bk* rolls) as in American practice they pass first to this intermediate-scratch-system.

Two other points of difference should be noted:

(1) Except for *A* purifier (purifying scalpings from first middlings coarse roll) it is not usual in British practice for reduction stocks to be purified. Thus, although there is a succession of purifiers operating on grades of break middlings, there is no exact equivalent to the American fourth purifier which may receive, as part of its feed, stock from the first tailings roll.

(2) The coarsest scalpings from the earlier coarse rolls invariably pass, in British practice, direct to the next coarse roll (e.g., B_2 tails to *F*, *F* tails to *J*), and not to offal as often in American practice, where only the second scalpings may be directed to the next coarse roll. This is important in the present discussion.

It should also be noted that it is difficult to equate exactly to British practice the fourth middlings roll, the feed to which appears generally to correspond partly with that to *E* and partly with that to *G* roll. We have bracketed *E* and *G* with the fourth middlings roll and put *H* as corresponding to the fifth middlings roll.

Operation of Mill. The principle (generally recognized in milling 80% flour) is followed at the Coxes Lock Mill of making very substantial releases early in both break and reduction systems, making as much semolina (coarse middlings) as possible, so that the roll surface allocation is in fact short for the early breaks and reductions but relatively long in the later stages. The later stages can therefore be used for careful selective action in the way of "cleaning up"; this is an important point. In the case of the reduction system this is helped by careful but heavy purification of well-graded semolinas (sizings)

and middlings, which enables heavy flouring to be done easily by the head reduction rolls. Scalping and grading for all breaks except the fourth, and sifting of *A*, *B*, and *C* reduction stocks (stocks from reduction of first and second middlings) is by Speight plansifter. *B* dunst, though good, is redressed on a centrifugal with advantage. The remaining reduction stocks and those from the fourth break are handled on centrifugals with covers ranging from 10 to 13 silk. There is a conventional *X* and *Y* scratch system with *X* and *Y* purifiers. *A* roll tails are purified. Tray separations, and in some cases tails, from middlings and fine semo purifiers, and from *X* and *A* purifiers, are dusted in a centrifugal, affording a small quantity of "TINS" flour, before passing to tins purifier, which grades stock to *E*, *F*, and *K* (fourth middlings, first tailings, and sixth middlings) rolls, respectively.

The mill is normally equipped throughout. It has 13 reductions (to *M*) of which B_2, *F*, and *J* ("second quality" or fine tailings, first tailings, and second tailings, respectively) are the customary second, third, and fourth quality coarse reductions (using the convenient terminology of J. F. Lockwood's discussion of the Reduction System in *Flour Milling*, 1945). *D* and *E* (third and fourth middlings) are fine rolls following *C* and B_2; *G* and *H* (fourth and fifth middlings) are the next group of fine rolls, following *F;* while *K*, *L*, and *M* (sixth, seventh, and eighth middlings or first low grade) are successive fine rolls following *J*. There are no detachers. Material from the last break, after treatment in the IV *Bk* purifier, passes along with that from *Y* purifier to each of the coarse rolls, B_2, *F*, and *J*. The IV *Bk* dunst goes to *K*, as also does "Tins" dunst. The only reductions having fluted rolls at the time of the test were *J* (the lowest grade coarse roll) and *L*, each of which had 40 flutes per inch and $2\frac{1}{2}$:1 differential. It should be noted that *L* also received pollards (approximately "shorts": second scalpings from the last break); in that way *L* and *M* include the functions of a Z_1 and Z_2.

Analytical Methods

The quantities of the individual flours liberated by each of these stages in the milling process (expressed as percentages of the wheat) are shown in the second column of Table I. These are based upon timings made by the mill staff. The last 10 columns of this table show the results of chemical analyses using the following methods:

Ash was determined by incinerating a 5 g sample overnight in a silica vessel at 600°C and the resulting ash weighed.

Fiber was determined by the method officially used for National flour in Great Britain as recommended by the Analytical Methods Committee of the Society of Public Analysts (1943).

TABLE I

COMPOSITION OF INDIVIDUAL MILL STREAMS CORRESPONDING TO NATIONAL FLOUR (80.5% EXTRACTION)
(Arranged in order of decreasing flour color; all results expressed on a 13% moisture basis)

Stream		Yield[2]	Protein (N x 5.7)	Fat[2]	Fiber	Ash	Thiamine	Riboflavin	Nicotinic acid	Iron	Manganese	Total phosphorus	Notes on flour. Color based on wetted Pekar slide
Description	American equivalent[1]	%	%	%	%	%	μg/g	μg/g	μg/g	mg/100 g	mg/100 g	%	
Group I. The Creamy Group (Patent Flour)													
A	Flour from 1st middlings coarse roll	10.4	10.7	0.93	0.10	0.40	0.54	0.40	9.0	0.69	0.30	0.082	*A* to *D* inclusive had a very similar creamy color, almost free from specks.
B	1st middlings fine	16.9	11.1	0.94	0.10	0.43	0.57	0.37	9.7	0.62	0.33	0.086	
C	2nd middlings	15.2	11.0	0.89	0.10	0.43	0.57	0.40	10.7	0.52	0.34	0.087	
D	3rd middlings	3.5	10.8	1.15	0.10	0.52	1.08	0.33	12.0	0.67	0.44	0.108	
		46.0											
B[3]	2nd quality roll or fine tailings roll	2.6	10.1	1.84	0.10	0.66	2.34	0.56	15.0	1.19	0.81	0.138	Slightly darker and greyer than above, color turns pale brown on wetting. Slightly specky.
Group II. The Greyish-Cream Group (Appreciably Specky). Just Slightly Better than National Flour													
Subgroup (A)													
E	Part of 4th middlings	2.0	11.7	1.23	0.10	0.60	1.74	0.53	13.5	1.00	0.64	0.125	*E* to *FMD* inclusive were distinctly more specky than those in group I but color was still creamy rather than greyish.
II *Bk*		3.5	14.0	1.25	0.18	0.63	1.02	0.60	18.0	1.10	0.54	0.131	
CMD[4]	Break middlings duster	3.5	12.4	1.29	0.15	0.65	1.68	0.68	19.4	1.18	0.59	0.132	
FMD[5]		2.9	12.4	1.28	0.20	0.66	1.53	0.61	13.5	1.18	0.62	0.133	
		11.9											
Subgroup (B)													
G	Part of 4th middlings	3.5	11.9	1.70	0.15	0.67	2.40	0.60	17.2	1.30	0.88	0.145	*G* to I *Bk* inclusive were slightly less creamy (greyer) and more specky than those in Subgroup A.
X		0.2	11.5	1.94	0.17	0.77	2.97	0.70	25.5	1.68	0.94	0.165	
I *Bk*		3.2	11.6	0.88	0.19	0.57	0.54	0.52	18.0	1.00	0.44	0.112	
		6.9											
National Flour (80.5% straight run) Pivotal line			12.0	1.43	0.202	0.724	2.67	0.69	19.2	1.41	0.99	0.154	

TABLE I—(*Continued*)

Stream		Yield[2]	Protein (Nx5.7)	Fat[3]	Fiber	Ash	Thiamine	Riboflavin	Nicotinic acid	Iron	Manganese	Total phosphorus	Notes on flour. Color based on wetted Pekar slide
Description	American equivalent[1]												
		%	%	%	%	%	μg/g	μg/g	μg/g	mg/100 g	mg/100 g	%	
Group III. Just Slightly Poorer in Color than National Flour													
Tins		0.2	11.7	1.46	0.37	0.74	1.65	0.64	24.6	1.84	0.72	0.145	Rather grey-brown but not very specky.
Group IV. The Brown Group													
Subgroup (A)													
F	1st tailings roll	1.2	12.3	2.45	0.24	0.97	4.05	0.92	25.1	2.19	1.55	0.211	*F* and III *Bk* were relatively dark, yellowish and specky. Color turns quite brown on wetting.
H	5th middlings roll	2.2	13.2	2.07	0.33	0.97	4.11	0.92	24.0	2.15	1.79	0.214	
III *Bk*		0.8	16.5	1.77	0.35	0.95	1.83	0.80	64.0	1.94	0.93	0.203	
		4.2											
Subgroup (B)													
Y		2.7	12.8	2.75	0.36	1.33	5.64	1.10	50.0	3.16	1.82	0.296	*Y* and IV *Bk* were rather darker but less coarsely specky than *F* and III *Bk*. Both exceptionally greasy.
IV *Bk*		0.9	16.0	2.54	0.41	1.82	2.85	1.44	84.0	4.04	1.59	0.400	
		3.6											
Group V. The Dark-Brown Group													
J	2nd tailings	1.4	17.7	5.35	0.97	3.24	32.40	4.00	32.9	8.50	8.27	0.730	All flours in this group were much speckier, dirtier, and browner than those in any of the above groups. *M* dunst redresser was much the worse; *K* was rather paler than the others.
K	6th middlings	1.2	15.6	3.65	0.90	2.17	16.20	2.60	56.5	5.53	4.96	0.483	
L	7th middlings	1.3	17.3	4.37	1.01	3.15	19.80	3.20	101.0	7.97	6.48	0.705	
M	8th middlings or 1st low grade	0.6	17.9	4.93	1.29	3.34	22.50	3.25	133.0	8.45	7.94	0.752	
M dunst redresser	2nd low grade	0.6	19.2	5.85	2.38	3.65	25.80	3.55	111.0	9.65	10.90	0.813	
		5.1											
Offals													
Coarse	Bran	7.1	12.4	3.86	10.85	5.78	4.95	2.89	296.0	11.80	12.20	1.172	
Fine		12.4	14.3	4.69	8.30	4.57	10.38	3.40	188.0	12.20	13.45	0.926	
Calculated weighted average of offals		19.5	13.6	4.39	9.23	5.00	8.40	3.21	227.0	12.1	13.00	1.014	
Calculated weighted average of all products		100.0	12.31	2.01	1.97	1.56	3.81	1.18	59.8	3.49	3.34	0.322	
Wheat (determined values)		100.0	12.37	2.03	1.97	1.51	3.87	1.28	57.2	3.14	3.41	0.321	

[1] The American equivalent term is given where different from English.
[2] Expressed as a percentage of the wheat represented.
[3] Petroleum ether extract.
[4] Coarse middlings dresser flour.
[5] Fine middlings dresser flour.

Fat was determined by extraction under a reflux condenser with boiling petroleum ether (B.P. 40°–60°C).

Thiamine was determined as recommended by Nicholls, Booth, Kent-Jones, Amos, and Ward (1942) and Booth (1942).

Riboflavin assays were conducted according to the method of Barton-Wright and Booth (1943).

Nicotinic Acid was determined by the method of Barton-Wright (1944).

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, A.O.A.C. (1940) and connected to protein by the factor 5.7.

Iron was determined by a method based on the work of Cowling and Benne (1942), Koenig and Johnson (1942), and Saywell and Cunningham (1937). The color of the ferrous o-phenanthroline complex was measured with a "Spekker" absorptiometer, using Ilford No. 604 filters and 4-cm. cells.

Manganese was determined as permanganate after oxidation with persulfate and silver nitrate. The method is based on the work of High (1945) and Richards (1930). Ilford No. 604 filters and 4-cm. cells were used.

Phosphorus was determined by the method of Allen (1940) with the substitution of 2 ml of 5*N* sulfuric acid solution for the 2 ml of 60% perchloric acid. Ilford No. 608 filters and 1-cm. cells were used.

The line next to the lowest in Table I shows the weighted average composition of all products, in comparison with the determined figures for the wheat set out in the bottom row. The comparison is clearer in Table II, which also shows the percentage deviations. The satis-

TABLE II

COMPARISON OF AVERAGE ANALYSES OF ALL PRODUCTS WITH VALUES DETERMINED ON WHEAT

Component	Determination on wheat	Weighted average of all products	Percentage difference [1]
Ash, %	1.51	1.56	+3.3
Fiber, %	1.97	1.97	0.0
Fat, %	2.03	2.01	−1.0
Thiomine, μg/g	3.87	3.81	−1.6
Riboflavin, μg/g	1.28	1.18	−7.8
Nicotinic acid, μg/g	57.2	59.8	+4.5
Protein, %	12.37	12.31	−0.5
Iron, mg/100 g	3.14	3.49	+11.1
Manganese, mg/100 g	3.41	3.34	−2.0
Total phosphorus, %	0.321	0.322	+0.3

[1] Calculated on the values for wheat.

factory nature of the sampling and timing (i.e., figures for percentage weights of the various streams) is shown by the fact that the deviations are spread each side of zero.

Iron Content of Flour

Having regard to the standards of accuracy in the various analytical methods the only serious, or, rather, significant, deviation is that of iron. This figure is known to be considerably affected through contact of materials with iron machinery, and it is conceivable, though perhaps surprising, that the 11% increase in iron content, from wheat to products, may be due to entry of iron particles and dust during processing. At first sight the chilled iron rollers of a flour mill might be expected to contribute to the iron content of roller-milled flour, since their working life is by no means indefinite.

According to Mr. J. F. Lockwood [2] of Messrs. Henry Simon, Ltd., in mills running 6,000 hours annually, fluted rolls were changed on the average every two years before the war, because wear on the edges of the teeth had altered the shape of the flutes. Rolls in mills with long surfaces might last 50% longer, short surfaces considerably less. Flute depths in common use vary between 0.0182 and 0.009 inch. No great increase in rate of wear has occurred under war conditions, except that reduction rolls fluted 35 and 40 per inch have required more frequent refluting, as they have frequently been set up in contact. Even in these cases the reduction in the roll diameter during work is not more than 3 or 4 thousandths of an inch per year. Reduction rolls with smooth surfaces run for very many years with a wear of less than three thousandths of an inch.

Assuming that the wear of a smooth reduction roll is 0.003 inch per annum (three one-thousandths in 10 years) it may be calculated that, for a reduction roll surface of 60 inches per sack, the liberation of metallic iron might raise the iron content of the total flour made by 0.007 mg per 100 g. It is rather more difficult to calculate a probable contribution from fluted rolls, but assuming that 0.0025 inch is actually worn off every flute per year, the contribution of iron from a total break roll surface of 40 inches per sack to the total flour milled would be only 0.0013 mg per 100 g.

Scrapers used on smooth rolls wear appreciably. In the experience of Mr. T. S. Parker, of Messrs. Henry Simon, Ltd., the wear amounts to $\frac{3}{8}$ inch in 20 years. Such scrapers average about $\frac{3}{32}$ inch in thickness. On this basis the contribution of metallic iron to the total flour milled would be only 0.0002 mg per 100 g, for 60 inches of smooth roll surface per sack.

The total contribution of metallic iron from all rolling operations is therefore unlikely to exceed 0.01 mg per 100 g. This is less than one thirtieth of the difference actually found in the present series between

[2] Private communication.

the wheat and the total products. The difference actually found, viz., 0.53 mg per 100 g, on the basis of 7000 lbs. per hour of wheat milled (as in the mill under discussion) would amount to $\frac{1}{40}$ lb. per hour of metallic iron or say $\frac{1}{2}$ lb. per day, or $1\frac{1}{2}$ cwt. per year. This seems large, but can hardly be ruled out as impossible, since much wear must take place in worm conveyors, elevators, spouts, and cleaning machines. It is these rather than the actual roller mills that would seem to be the main contributors of metallic iron to the flour and offal.

It is also conceivable that the discrepancy originates from fragments of iron, entering with the grain, which pass into the screenings. These might be missed in sampling the wheat but would be reintroduced to the Fine Offal with the ground screenings.

Color and Nutrient Content of Streams

The main commercial consideration in flour has always been that of color and in Table I the various flours have been arranged in that respect. "Color," as discussed here, is that of the *wetted Pekar slide*, as customarily used in commercial practice, and not the "color score," which has proved so useful in classifying long-extraction (straight-run) flours. The latter is a measure of content of discrete bran specks, and is applied to straight-run flours from different mills. It is not directly applicable to the present series, where the individual streams vary greatly in degree of fineness of the contained bran. The order of arrangement in Table I is quite familiar to the miller, who ordinarily looks to the head reduction rolls, *A*, *B*, and *C*, to furnish half the total output of flour, in the form of patent flour: that is, the flour of creamiest and cleanest color (freest from bran specks). The next group of flours listed adds just over a third, making altogether 84%, of the total output of flour, and has color appreciably and progressively poorer (more greyish, more specky) than the patent group, but none of its members is poorer than the National, or 80% straight run, flour (see the "Pivotal Line" in Table I). Below this line lie the "brown" and the "dark-brown" groups, making about 10% and 6% respectively of the total flour output. Of these the dark-brown group in entirety would have entered the weatings or offals in prewar white flour milling, while some of the brown group would not have been quite so brown, principally because less severe work would have been done in the earlier parts of the milling system. Moreover, for the same reason, the upper groups themselves would have been fractionally better in color and lower in ash content. The top three flours (*A*, *B*, and *C;* making up about half the total flour) are, as a group, from 0.05% to 0.1% higher in ash than corresponding flours from ordinary prewar (70% extraction) milling. Most of the remaining flours, down to

Group 3 in Table I, are higher in ash, by between 0.1 and 0.2%, than corresponding prewar flours. Thus a flour corresponding to 70% of the wheat, made by excluding the darkest 10.5% of the flour streams of Table I, should by calculation contain 0.49% ash, which is almost 0.1% higher than that of a good prewar 70% flour. A 70% flour actually made a little later in this way, by excluding the bottom 10% of the National flour, gave the following analytical figures:

Ash	0.51%
Thiamine	1.05 μg/g
Fiber	0.15%

Table I shows how well the ash content corresponds with the relative placing of the flours according to color. This is not surprising, for ash content has been long recognized as an effective means of characterizing various grades of flour in commerce. Only two flours are really out of place in the double ranking (i.e., according to color *and* to ash content), viz. B_2, roll flour and the first break flour. B_2 is exceptional in being the first of the tailings or lower grade coarse rolls, i.e., it operates on a feed containing a fair amount of germ which has been flattened in the preceding higher grade reductions. B_2 roll flour therefore is the first flour to contain an appreciable proportion of germ. At the same time, its speckiness or bran content is slight because the feed to B_2 roll, though germy, is not very branny—hence the rise in thiamine, fat, riboflavin, nicotinic acid, and ash, etc. (in all of which the germ is more or less rich)—while the fiber content (reflecting bran content) is not appreciably increased. The first break flour is opposite in that it has lower ash content than its appearance would indicate. This flour contains substantially no germ, as indicated by the very low thiamine figure, but does contain an appreciable amount of bran powder—shown by the rise in the fiber figure, which, incidentally, is in the right place in the ascending order of the Table.

Table I shows that, in general, the content of nutrients rises with falling color (and also with falling baking quality, discussed in a later section). The dark-brown group as a whole is much the richest in nutrients. In certain general characteristics the trend accords with that found by Jackson, Doherty, and Malone (1943) in their analyses of the mill streams of a large Canadian mill, which was milling all-Manitoba wheat to a flour extraction of 74%. Their riboflavin and nicotinic acid values for the wheat itself were very similar to those of the present wheat mixture, but the thiamine content was higher (4.53 as against 3.87 μg/g). The ash content of their various grades of flour, up to a total extraction of, say, 70%, ran lower than the present values substantially in the manner described above. Despite this, their nicotinic acid values for the patent flour (top 37% of

wheat), for the next grade (first baker's, bringing the total length to 63%) and for the third grade (second baker's, to 70%) were, respectively, very similar to those shown in Table I of this article for corresponding groups of flours. On the other hand their thiamine and riboflavin figures were both relatively higher in the top (patent) grade but lower (thiamine, considerably; riboflavin, slightly) in the two lower grades.

In Table I the flours from the tailings, or lower quality coarse, rolls show sudden steps upward in thiamine content—at B_2, F, and J—because these are points of concentration and re-entry of germ. With a system entirely of smooth rolls, the flours from the group of fine reductions immediately following a given coarse roll would be expected to show no downward trend in thiamine, because the dunsts from the coarse roll feeding them would also be rich in germ. Thus H flour is as high as F flour in thiamine content. Usually, however, there is dilution of the feed to such fine reductions with fresh material from the break system or from earlier fine reductions. For example, G also receives fine middlings from the early breaks and dunst from E, which lower the nutrient content of its flour (and as every milling student knows, improve its appearance or grade). K is similarly affected, relatively to J, by dilution with dunst from the fourth break and from H. When, however, a low-grade coarse roll is fluted (cf. J roll in the present series), its flour shows a markedly high thiamine figure, owing to the greatly increased breakdown of germ at that point.

The break flours generally are very low in thiamine content relative to their color position and (possibly apart from the last break flour) contain practically no germ. Their high content of certain nutrients is due to their progressively increasing content of bran (as reflected in the fiber figure) and, more important, of endosperm from the peripheral portions of the grain. This endosperm has been shown (Moran, 1945) to be high in iron, nicotinic acid, and protein. This explains the fluctuations in the nicotinic acid figures in Table I. Outer endosperm enters, progressively more markedly, into the II, III, and IV break flours, and to some extent (as would be expected since scratch rolls resemble fine break rolls) into the scratch roll flours X and Y. It also affects the nicotinic acid value of L flour because, as mentioned, this roll receives pollards ("shorts" or fine bran), and it appears that, possibly partly for this reason, the last reduction, or M and M re-dresser, flours contain considerable proportions of outer endosperm. The nicotinic acid content of bran itself is relatively very high and this itself accounts in part for the nicotinic acid levels at the points mentioned.

The level of protein content is interesting in that it is affected in two ways. The outer endosperm is relatively high in protein content and the germ is exceptionally high (over 30%). The first factor accounts for the quite high level (16-16.5%) reached in the third and fourth break flours, while the second accounts for the very high level of the very germy flours, e.g., *J* (17–18%). When outer endosperm appears to be present as well as germ, the figure becomes higher still, as in *M* redresser flour (19.2%).

Riboflavin appears on the whole to be well related to ash content (and to order of color).

The fat data deserve consideration especially in connection with those for thiamine. To begin with it appears that the outer endosperm must be very high in fat content. This must be assumed, in order to account for the relatively high fat contents of the later break flours which contain little or no germ. It is again in keeping with the high figure (the highest fat content shown in Table I) for the *M* redresser flour, already assumed to contain outer endosperm.

Germ itself contains at least 10% of fat (extracted with petroleum ether). Table I indicates that the main or inner endosperm from this grist, in relatively pure condition, has a fat content of about 0.9%. One would expect therefore the excess over 0.9% of the fat content of any given flour to be connected with the proportion of germ contained in it, and therefore to its thiamine content. So far, however, nothing has been said about the two subdivisions of the germ, viz., the scutellum and embryo. In this paper the term "embryo" will later be used, purely for convenience, to denote what should strictly be termed "the part of the embryo other than scutellum" (i.e., the dormant root, stem, and plumule). As shown by Hinton (1943, 1944) the former is the main seat of thiamine in grain, having a thiamine value of about 150 μg/g while the embryo has only about 9 μg/g. It is probable that the scutellum contains more fat than the embryo (Hinton, 1944), but the fat content of both is high. Any flour which contains much more scutellum than embryo should therefore have a relatively high ratio of thiamine to fat, and vice versa. It is interesting to inspect Figure 1, which shows the thiamine values for the various samples of Table I, plotted against fat contents. Only in a general sense does thiamine rise with fat content, as shown roughly by the straight line drawn cutting the horizontal axis, as seems natural at about 0.9%. Points for some flours lie well above and some below this line. It is suggested that the former flours contain a more or less high ratio of scutellum to embryo, and the latter a low ratio. The outstanding case, of course, is *J* where the ratio of scutellum to embryo must be particularly high. It is significant that *J* is the first *fluted* roll in the reduction system of

this mill. This confirms the value of finely fluted rolls for reducing scutellum, as stressed by Kent *et al.* (1944). The graph also indicates that each of the flours from the tail end of the reduction system (*K* and onwards) is rich in scutellum relative to embryo, while the reverse is the case with the germy flours from earlier in the system, e.g., B_2 and *F*. It would be very interesting to see where the point in the graph for *F* flour would lie in a similar mill having fluted *F* rolls.

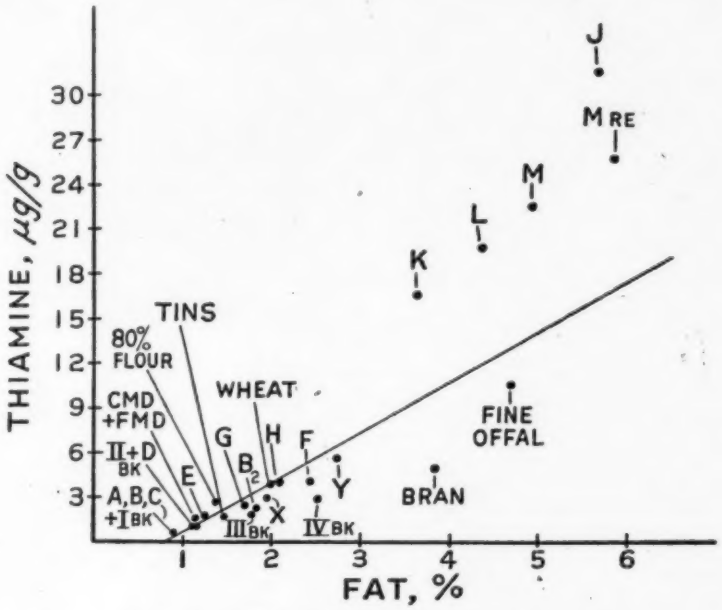

Fig. 1. Relation between the thiamine and fat content of flour streams composing National flour of 80.5% extraction.

The phosphorus and iron content follows the ash content closely (Table I). Manganese, however, follows the germ content more particularly, which is to be expected, since W. J. S. Pringle in these laboratories finds that the manganese content of the grain is relatively highly concentrated in the germ. Thus in the present data though the proportion of manganese is less than half the iron in the pure endosperm (e.g., *A* flour), yet it tends to reach or even to exceed the figure for iron in the germy flours.

Baking Tests

Baking tests were made employing absorptions determined with the instrument developed by Halton and Fisher (1937). These absorptions (calculated to a 14% moisture basis) and the heights of the 1-lb. loaves baked are shown in Table III. The system used 4 hours'

TABLE III

RESULTS OF BAKING TESTS ON INDIVIDUAL FLOURS COMPRISING NATIONAL FLOUR OF 80.5% EXTRACTION
(Arranged in order of decreasing crumb color)

Flour	Baking tests without potassium bromate				Baking tests with potassium bromate			
	Water[1] absorption	Loaf[2] height	Dough quality	Crumb color	15 ppm. $KBrO_3$ added general loaf quality	Bromate tolerance: Total bromate	Bromate tolerance: Loaf height[2]	Notes on response
	%	*Inches*				*ppm.*	*Inches*	
Group I. Pale Cream Crumb								
A	58.6	4.9	All flours in this group yielded good doughs with fairly good to good extensibility and spring.	*A* and *C* gave similar pale cream crumb colors. *B*, *D*, and *E* gave progressively slightly deeper color.	*A* and *C* excellent with close even grain and smooth texture. *B*, *D*, and *E* very like above but crumb slightly less regular. *B* had slightly firmer crumb than others.	15		
C	59.6	5.0				15		
B	57.8	5.0				15		
D	64.3	4.9				15		
E	65.3	5.1				15		
Group II. Deep Cream Crumb								
B_2	59.6	4.4	Generally good doughs with good extensibility and spring and with but slight stickiness except B_2 and *X*. B_2 gave a very sticky, slightly short dough with only fair spring. *X* gave a very sticky dough. I *Bk*, II *Bk*, *CMD*, and *FMD* gave very extensible doughs.	Progressively slightly deeper color with greyish-brown tinge becoming more noticeable, *G*, *X*, and Tins being considerably darker than the others. Even B_2 distinctly deeper and more brownish than *E* in top group. *G* to Tins gave softer and better developed crumbs than B_2 to II *Bk* but of poorer color.	B_2 to II *Bk* inclusive alike in crumb which was rather less even but firmer and less well developed and with a rougher texture than group I.	20	4.6	Only slight increase in loaf volume. I *Bk* to II *Bk* inclusive gave large volume increase with slightly open and irregular crumb, but of good spring.
I *Bk*	55.7	4.6				20	5.2	
FMD	57.5	4.5				20	5.2	
CMD	56.5	4.6				20	5.2	
II *Bk*	58.2	4.7				20	5.2	
G	62.5	4.6				20	5.2	Large volume increase, very soft crumb of good spring.
X	56.8	4.4				20	4.8	Slight volume increase, bromate addition gave better grain and spring of crumb.
Tins	58.2	4.9				20	4.9	No response: crumb rather weak.

TABLE III—(*Continued*)

Flour	Water[1] absorption	Loaf[2] height	Baking tests without potassium bromate: Dough quality	Baking tests without potassium bromate: Crumb color	Baking tests with potassium bromate: 15 ppm. $KBrO_3$ added general loaf quality	Bromate tolerance: Total bromate	Bromate tolerance: Loaf height[2]	Notes on response
	%	*Inches*				*ppm.*	*Inches*	
Group III. Pale Brown Crumb								
III *Bk*	60.0	4.4	All doughs were very extensible but sticky. All gave good spring except *H*. III *Bk* gave particularly good spring.	III *Bk* and *F* similar (pale brown).	III *Bk* to *H* gave smaller loaves with denser crumb than group II. *H* rather more open and softer than III *Bk* and *F*.	25–35	5.4	Marked volume increase; well-developed soft but rather irregular crumb.
F	61.5	4.5				25–35	4.7	Some volume increase, better developed; crumb much improved including color.
H	71.5	4.3		*H* slightly browner and *Y* and IV *Bk* markedly browner.		25–35	4.7	Some volume increase, better developed; crumb much improved including color.
Y	56.8	3.9			*Y* and IV *Bk* gave still smaller and denser loaves.	25–35	4.5	Great improvement in volume and development. Crumb improved but still close in grain.
IV *Bk*	61.5	3.8				25–35	5.2	Outstanding improvement in all characters.
Group IV. Dark Brown Crumb								
K	66.1	2.8	Very unsatisfactory doughs, progressively very short, sticky and clay-like.	Progressively poorer color from *K* to *MRE* (*MRE*, a dirty grey-brown).	All flours gave very small, bound and dense loaves.	35–55	2.8	All flours in this group gave little volume increase but there was some improvement in the crumb.
L	64.3	2.8				35–55	2.8	
J	62.9	2.4				35–55	2.8	
M	67.9	2.4				35–55	2.8	
MRE	84.3	2.4				35–55	2.8	
National Flour	61.8	5.0	Good extensibility and spring, slightly sticky.	Flour was bleached; gave a greyish-white crumb color.	Larger loaf and more open and woolly than groups I to IV inclusive.			

[1] Expressed on 14% moisture basis.

[2] Height of 1 lb. loaf (tinned).

fermentation (including final proof) at 80°F (26.7°C), with 1.3% yeast and 1.8% salt. The doughs were made and molded by hand throughout.

The samples are arranged in Table III according to the color of the loaf crumb. They fall into four broad groups (the second of which consists of two subgroups), well differentiated from one another. Group 1, with pale cream color, and Group 2, with deep cream crumb color, substantially reproduce the order of the first two groups of Table I (which was based upon flour color), except that (1) *E* flour comes up into the top group and B_2 moves down to the second group; (2) the order of the I and II *Bk* flours is interchanged; (3) the tins flour is brought up into Group 2. Group 3, in Table III, corresponds with the brown group of Table I; the III *Bk* flour, however, comes out at the top of this group on the score of crumb color. Group 4 includes members of the dark-brown group of Table I, none of which makes anything approaching acceptable bread when baked alone.

The last three columns in Table III are of special interest as showing the different responses to improver treatment of these various components of the National flour. The first two groups require no more than light treatment and of their members the early break flours (and middlings dresser flours, which are really break flour), *G* and *X* show considerable response. The third group as a whole requires heavy treatment and responds markedly (the fourth break flour, as is well known, being outstanding in its response to improver treatment). The members of the fourth or dark-brown group show slight improvement and require very heavy dosage.

This work has been carried out on a mixed grist. Though no consideration of possible segregation of the components (Manitoba and English) in the milling process has been attempted, it is believed that the differences recorded are due mainly to the interplay of the different parts of the grain in the ways discussed rather than to segregation of particles from hard and soft wheats.

Summary

Proportions and composition (protein, ash, fiber, fat, thiamine, riboflavin, nicotinic acid, iron, manganese, and phosphorus) are given for each of the 23 individual flours which compose a National flour of 80% extraction and good color, and for the corresponding offals. The weighted averages agree well with the corresponding figures for the wheat (60% Manitoba, 40% English), except in the case of iron, where the amount in total products exceeds that in the wheat by 11% of the value; considerations of known extents of wear indicated that this

entry of iron must have occurred in a part of the plant or process other than the actual roller-mills.

The special measures generally adopted in milling 80% flour in Britain are briefly described, regard being given to differences between American and English technical terms. The streams composing the best (or "patent") half of the 80% flour have 0.1% fiber content and ash content not exceeding 0.43%: this ash content is from 0.05 to 0.1% higher than that of corresponding flours from ordinary 70% extraction milling. The difference becomes greater with the lower grade flour streams.

The ash and fiber contents of the flour streams in general accorded well with the placing according to color. The best 12 of the streams, amounting to 84% of the total flour, were all superior or equal to the National or 80% straight-run flour in color and, generally, lower or not higher in content of ash, fiber, B vitamins, iron, manganese, and phosphorus. When untreated or with only light improver treatment, these flours had the best baking quality. The remaining flour streams fell substantially into two groups, the brown and dark-brown, comprising 10% and 6% respectively of the total flour, with markedly rising contents of ash, fiber, and all nutrients. The latter group, much the richer in nutrients, would in 70% extraction milling pass entirely to offal while some members of the brown group would have been less brown. Members of the dark-brown group, individually, have very poor baking quality, though in the total flour they are "carried" by the general strength of the grist. They show very great tolerance to improvers, as exemplified by bromate, though the response is disappointingly small. Tolerance is less, though still great, in the brown group but here the response is marked.

The thiamine values for the series of streams show marked increases in the flours from the tailings, or low-quality coarse, rolls because these represent points of concentration of germ. These increases are particularly marked when such rolls are fluted (in accordance with present general British practice); for example, in the present series the *J*, or second tailings, roll was the first fluted reduction roll and the flour sifted from its grind (*J* flour), amounting to 1.4% of the wheat, had a thiamine content exceeding 30 μg/g

The break flours contain little or no germ. Their relatively high content of some nutrients, especially nicotinic acid—progressively greater in approaching the later breaks—is due to increasing content of bran powder, and, more important, of outer endosperm. This, while not particularly rich in thiamine, is very rich in nicotinic acid, protein, iron, and fat. The later break flours are thus high in protein and in fat because they contain much outer endosperm, while the later

reduction flours are high in protein and fat because they contain much germ. In the last reduction (second low-grade) flour of the present mill, both germ and outer endosperm are present and protein and fat both reach maximum values.

In this series of flours the relationship between fat and thiamine content is not a close one. This is to be expected, not only from the disturbing effect of the outer endosperm (rich in fat but not in thiamine), but also because thiamine is mostly concentrated in the scutellum, while both scutellum and nonscutellum parts of the embryo are rich in fat. The latter enters preferentially into some of the flour streams whereas in others, particularly towards the end of the reduction system, the scutellum enters preferentially.

Any or each of the three—germ, outer endosperm, and bran—may contribute to the ash content of the individual mill streams. The riboflavin, phosphorus, and iron values follow the ash contents, but the manganese values follow more particularly the contents of germ.

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USE OF SPRAY-DRIED WHOLE-EGG POWDER IN SPONGE CAKES [1]

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The purpose of this investigation, one of a series on the use of spray-dried whole-egg powder in cookery, was to study the leavening property of dried eggs and to develop a practicable method, if possible, for making acceptable sponge cakes from the whole-egg powder.

In sponge cakes, eggs serve as a leavening agent by incorporating and retaining air when beaten. They exert a binding effect, serving to maintain the cell structure of the batter during the baking process, and thus helping to impart lightness and characteristic texture to the cake. They also give flavor and color to the cake (Bennion, Hawthorne, and Bate-Smith, 1942).

The leavening power of eggs depends not only upon the amount of air which can be incorporated during whipping, but also upon the strength of the foam produced (Hawthorne and Bennion, 1942). A foam of suitable stability, if deftly handled while the flour is being incorporated, allows the air to remain in the batter during the final mixing and baking, and the resulting cake will be light, tender, and of good volume (Pyke and Johnson, 1940). If the foam is not sufficiently strong to support the flour, the finished cake will likely be less tender and have smaller volume (Hawthorne and Bennion, 1942).

Since the age and quality of eggs, the temperature of beating, and other factors affect the rate at which egg-sugar foams or meringues are formed when eggs and sugar are beaten together, it has been suggested that specific gravity determinations and not time should be used to indicate when a batter is beaten sufficiently if cakes of high

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quality and uniformity are to be obtained (Pyke, 1941; Pyke and Johnson, 1940, 1941).

A few reports on the whipping and baking properties of dried whole-egg powder have appeared in the literature. Bennion, Hawthorne, and Bate-Smith (1942) found a correlation between the volume of the egg-sugar foam and the quality of the sponge cakes baked from the foam. They reported that, in general appearance and volume, cakes made with dried egg plus a secondary aerating agent were only slightly inferior to cakes made from frozen eggs. They concluded that by slight alterations made in usual baking procedures, good quality spray-dried whole-egg powder, properly prepared and stored, could be used to produce sponge cakes of medium to good quality, even without the addition of a second aerating agent. They observed that better results were obtained when a temperature of 100°F (37.8°C) was used for beating the egg-sugar-water mixture than when the beating was done at 65°–70°F (18.3°–21.1°C). Hawthorne and Bennion (1942) made a more extensive study of the influence of temperature upon the beating and baking properties of spray-dried egg. They found considerable improvement resulting from the use of higher temperatures than are usually employed in commercial practice. Both the volume of the foam and the rate of formation of the foam were increased by beating the reconstituted egg with sugar at the higher temperature. Using a temperature of 104°F (40°C), 5 to 10 minutes of beating yielded as large a volume of foam from a given egg sample as 20 to 30 minutes beating at 64°F (17.8°C). In some cases, still greater improvement in the formation of the foam resulted from using temperatures around 120°–122°F (48.9°–50°C) for whisking, provided additional water was added to the egg-sugar mixture before mixing to compensate for evaporation during beating. These investigators concluded that, by choosing suitable conditions, it should be possible to make first-quality sponge cakes from almost any sample of spray-dried egg, although some special equipment might be necessary in bake shops.

Woodcock and Reid (1943) used the volume of sponge cakes made from various egg powders as one measure (among many others) of assessing the quality of powders prepared under different drying conditions.

Reid and Pearce (1945) in a study of the relationship of various objective tests to baking quality of dried whole-egg powder found a high correlation ($r = + 0.96$) between foaming volume of the powders and loaf volume of small sponge cakes baked from the same powders, when prepared under the conditions of temperature and humidity used in their experiments.

Materials and Procedure

For the first exploratory work in which 83 cakes were baked, the egg powder used was spray-dried whole-egg powder of good quality which conformed to the 1944 specifications of the War Food Administration as to moisture content. For the remainder of the study, there was available low-moisture powder which was produced according to 1944 U. S. Army specifications. This low-moisture powder came packed in No. 10 tin cans, and each was labeled with the date of drying. As soon as the egg powder was received in the laboratory, the date of receipt was recorded and the powder was placed at once in a refrigerator maintained at about 40°F (4.4°C). When a can of the powder was opened, the contents were placed in quart glass jars and tightly covered. The egg powder was kept in the refrigerator until used.

The other ingredients used in each series of cakes were as nearly identical as possible. The granulated sugar for all the cakes came from the same lot, as did also the salt; cake flour of the same brand was used throughout, and for any given series it came from the same lot. Tap-water was used for reconstituting the egg powder.

The temperature of the ingredients was controlled as much as feasible. Records were kept of the temperature of the laboratory, of the foam, and of the batter.

Objective measurements made included the specific gravity of the egg-sugar foam and of the finished batter; the weight and volume of each cake; and the tensile strength of slices from the cake. The volume was determined by seed displacement, using a method similar to that described by King, Morris, and Whiteman (1936). The tensile strength determinations were made with an apparatus similar in design to the one used by Pyke and Johnson (1940). Slices used for tensile strength measurements were cut from the center of the cake; each was cut 2 cm thick and trimmed and cut into an hour-glass shape, the width of the center of which was 2 cm. The tensile strength readings were recorded as grams of force required per square centimeter of cross-section area to break the slice of cake.

The pH of the batters was determined in one series of cakes, a Beckman pH meter with glass electrode being used.

The beating and mixing were done for the most part on an electric mixing machine, using a wire whisk and 4-quart bowl. In the exploratory work, an electric mixer with the blade type rotary beater was used, but, since the mixer with the wire whip seemed to produce a better foam and required less time for beating, it was used in all later experiments.

The cakes, except in a few special cases, were baked in pans

$3\frac{5}{8} \times 5\frac{5}{8}$ inches at the bottom, $4\frac{1}{8} \times 6$ inches at the top, and $2\frac{1}{2}$ inches deep. The weight of batter baked in each pan was 175 g. Thermostatically controlled, ventilated gas ovens were used for baking.

In the exploratory experiments, the effect of different proportions of flour and sugar (within ranges found in standard recipes for fresh egg sponge cakes) to a given weight of egg powder was noted; the effect of various initial temperatures of ingredients upon the speed of foam formation was studied; and the specific gravity of foam and of batter which produced the better cakes was observed. As a result of these observations the following formula was tentatively set up for use in more detailed studies: Cake flour, 40 g; granulated sugar, 70 g; whole egg powder, 26.2 g; salt, 0.5 g; cream of tartar, 0.5 g; water, 95 to 105 ml.

Series I. In this series of more than 120 cakes, the following variables were studied: Three levels of water (95, 100, and 105 ml); two baking temperatures (375°F [190.6°C] for 19 minutes, and 400°F [204.4°C] for 15 minutes); and five levels of cream of tartar (0.0, 0.5, 1.0, 1.5, and 2.0 g).

The relative quality of the cakes was judged organoleptically by observing the grain, lightness, moistness, and flavor of samples; and objectively by the measurements of volume and tensile strength. Analyses of variance of the data were made in order to determine the relative importance of the variables used in the formula and in the manipulation. The correlation coefficients between specific gravity of the finished cake and that of the foam and of the batter were calculated.

Having established, through the experiments in Series I, some of the conditions for making an acceptable sponge cake from egg powder, still further improvements were sought. A modified sponge cake formula, including baking powder (a cream of tartar baking powder), gave promise of some improvement. Hence, a series of cakes was baked studying this factor.

Series IIa. A factorial experiment was set up in which three levels of cream of tartar and three levels of baking powder (0.0, 0.5, and 1.0 g) were used in all combinations, making a total of nine formulas. Since it was not feasible to bake more than five cakes in one baking period, an incomplete block design was used for the experiment. Ten replications of each cake were made; there was a total of 18 blocks of five cakes each, and each cake was baked in a period with every other cake five times. The order of the blocks and the order of mixing cakes within a block were selected at random. Determinations of volume and of tensile strength of the baked cakes were made and the data analyzed for statistical significance.

The formulas and the method of mixing for this series of 90 cakes were as follows:

BASIC FORMULA FOR SPONGE CAKE

Flour, cake	40 g
Sugar, granulated	70 g
Dried egg powder, low-moisture	26.2 g
Salt	0.5 g
Water, tap	95 ml
Lemon extract, ¼ teaspoon	

The combinations of cream of tartar and baking powder were as follows:

Key to formula	Cream of tartar	Baking powder
	g	g
B	0.0	0.0
C	0.0	0.5
D	0.0	1.0
E	0.5	0.0
F	0.5	0.5
G	0.5	1.0
H	1.0	0.0
A	1.0	0.5
I	1.0	1.0

The egg powder used for this series was dried in February, 1945, and used during March, April, and May following.

The procedure for mixing and baking was as follows:

(1) The sugar, egg powder, salt (and cream of tartar, if used) were sifted together into the mixing bowl which was placed in a pan of hot water, double-boiler fashion. These ingredients were mixed and stirred until warmed to a temperature of 104°F (40°C).

(2) The 95 ml of water at a temperature of 176°F (80°C) were added all at once to the dry ingredients in the bowl and the mixture stirred lightly. The temperature of the resulting mixture was near 140°F (60°C).

(3) The mixing bowl was at once attached to the electric mixer and whipped (using wire whip attachment) at high speed for 30 seconds. The flavoring was added, and the whipping continued at high speed until the meringue reached a specific gravity of 0.270 to 0.306. (The time necessary to reach this point varied from 3.5 to 7.5 minutes with an average of 4.87 minutes.) The temperature of the meringue at the end of the beating period was usually about 86°F (30°C).

(4) The flour (and baking powder, if used), sifted once, was added all at one time to the egg-sugar foam. The mixer was started at low speed, and the flour was mixed into the foam for a period of 30 seconds. The mixer was stopped, the bowl scraped down, and then the mixing continued at low speed for another half-minute. The mixing was such that the specific gravity of the batter was 0.342 to 0.378.

(5) The batter—175 g—was poured into an ungreased pan.

(6) The cake was baked for 19 minutes in a 375°F oven.

The cake was cooled and left in the inverted pan overnight. The following day the volume and tensile strength measurements were made.

Series IIb. When the pH meter became available for use, a series of 45 cakes was baked, five cakes being made by each of the nine formulas used in the series of 90 cakes. The pH of the batters was taken in order to note any relationship which might be apparent between pH of batter and quality of the cake. The egg powder used in this series was dried in April, 1945, and used in July following.

Supplementary Group. A group of miscellaneous cakes was baked in which ingredients were measured instead of weighed, and in which some cakes of family size were baked. Some fresh egg cakes were baked for comparison.

Results and Discussion

In the preliminary work, it was observed that if the temperature of the egg-sugar-water mixture was around 140°F (60°C) at the beginning of the beating period, a lighter and more stable foam was formed and a much shorter beating time was required than if the ingredients were at room temperature, 70°–77°F (21.1°–25°C). This observation was in agreement with the findings of Hawthorne and Bennion (1942).

The cakes in Figure 1 illustrate the effect of temperature and time of beating the foam upon the quality of the finished cake. Cakes 1, X, and Y were made with the same proportion of ingredients. The egg-sugar-water mixture for Cake 1 was at a temperature of 140°F (60°C) when beating was started and the time of beating required to produce the foam of satisfactory specific gravity was 5.5 minutes. For Cake X, the egg-sugar-water mixture was beaten at room temperature for 5.5 minutes, and, in that length of time, no satisfactory foam was produced; consequently, the cake was a failure. For Cake Y, the egg-sugar-water mixture was beaten at room temperature for 15 minutes, but, even after that length of time, the foam was not as light as for Cake 1 and, consequently, the resulting cake was not of as great volume. The formula for Cakes 1, X, and Y included 1.0 g each of cream of tartar and baking powder. The formula for Cake Z had 0.5 g cream of tartar, but no baking powder; the egg-sugar foam was beaten at room temperature for 25 minutes.

The effect of other variables in the formula and procedure was studied. From analyses of variance made on data from groups of cakes baked in Series I, using tensile strength measurements as the criterion of quality, it was found that the variance due to three levels

of water—95, 100, and 105 ml—used in the formula, made no statistically significant difference in the cakes. The same was true of the two baking temperatures, 375°F (190.6°C) for 19 minutes and 400°F (204.4°C) for 15 minutes. The effect upon tensile strength of using different levels of cream of tartar, varying from 0.0 to 2.0 g, however, was highly significant (beyond the 1% level of significance). By organoleptic tests, cakes made with 95 ml water and 0.5 to 1.0 g cream of tartar in the formula, and baked at 375°F (190.6°C) for 19 minutes were generally judged to be preferable.

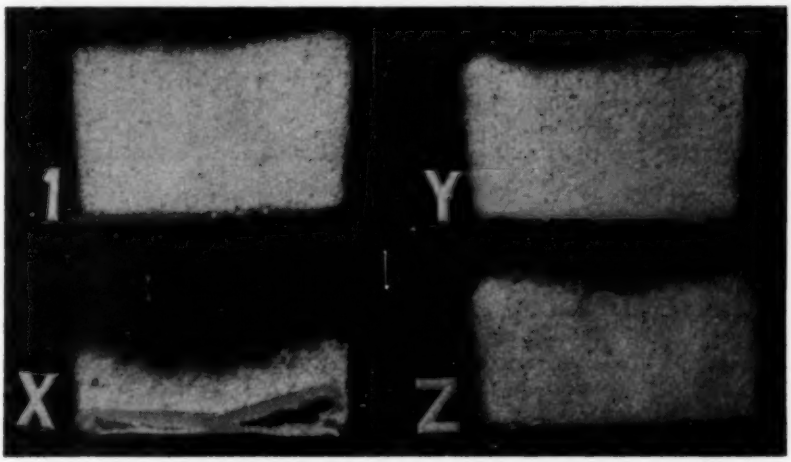

Fig. 1. Effect of initial temperature of egg-sugar-water mixture and time of beating upon quality of cakes made from dried egg powder. Cakes 1, X, and Y were made by the same formula which included 1.0 g each of cream of tartar and baking powder. The formula for Cake Z included 0.5 g of cream of tartar, but no baking powder.

	Initial temperature of egg-sugar-water mixture °F	°C	Time of beating the foam *min*
Cake 1	140	60.0	5.5
Cake X	75	23.9	5.5
Cake Y	75	23.9	15.0
Cake Z	75	23.9	25.0

The coefficients of correlation between the specific gravity of the baked cake and that of the egg-sugar foam or meringue, and that of the batter, were 0.63 and 0.77, respectively. These coefficients were based upon records from 138 cakes. Both of the values were statistically highly significant. A lower coefficient of correlation between batter and cake might be expected, since a light foam may not always be sufficiently strong to support the flour (Hawthorne and Bennion, 1942).

In Table I are given the averages of the measurements on the cake batters and finished cakes made in Series IIa and IIb. The 45 cakes in Series IIb were baked from a different lot of egg powder and under

TABLE I

AVERAGE RESULTS OF MEASUREMENTS ON THE CAKE BATTERS AND FINISHED CAKES OF SERIES II

Key symbol	Cream of tartar g	Batter			Finished cake		
		Specific gravity		pH	Specific gravity	Specific volume [1] cc/g	Tensile strength [1] g/cm²
		Egg-sugar foam	Batter				
SERIES IIa—90 cakes							
0.0 g baking powder							
B	0.0	0.286	0.376	—	0.190	5.26	30.07
E	0.5	0.294	0.365	—	0.186	5.37	29.79
H	1.0	0.294	0.373	—	0.184	5.43	25.06
0.5 g baking powder							
C	0.0	0.297	0.372	—	0.182	5.49	28.92
F	0.5	0.297	0.366	—	0.180	5.56	28.25
A	1.0	0.294	0.359	—	0.179	5.58	24.59
1.0 g baking powder							
D	0.0	0.291	0.365	—	0.175	5.71	26.84
G	0.5	0.296	0.355	—	0.173	5.78	25.88
I	1.0	0.295	0.353	—	0.176	5.68	22.73
SERIES IIb—45 cakes							
0.0 g baking powder							
B	0.0	0.280	0.365	7.65	0.191	5.23	25.16
E	0.5	0.288	0.364	6.29	0.191	5.23	25.68
H	1.0	0.290	0.362	5.50	0.190	5.27	22.32
0.5 g baking powder							
C	0.0	0.278	0.352	7.38	0.185	5.40	23.95
F	0.5	0.277	0.345	6.40	0.182	5.49	23.25
A	1.0	0.283	0.352	5.64	0.185	5.40	21.45
1.0 g baking powder							
D	0.0	0.284	0.354	7.22	0.179	5.58	22.72
G	0.5	0.287	0.342	6.41	0.178	5.61	24.42
I	1.0	0.293	0.351	5.66	0.183	5.46	21.99

[1] Least difference in means necessary for significance in Series IIa (90 cakes):

	Specific volume	Tensile strength
At 5% level.....	0.14	1.46
At 1% level.....	0.18	1.94

different laboratory conditions of temperature and humidity from the 90 cakes of Series IIa, yet the results were very similar.

Tensile strength measurements are an indication of relative tenderness; the lower the tensile strength, the more tender the cake. Cakes made from formulas A, H, and I, containing 1.0 g of cream of tartar, were the most tender both in Series IIa and IIb, as indicated by the lower tensile strength values. An analysis of variance on data from the 90 cakes indicated that both the cream of tartar and the baking powder influenced the tensile strength measurements to a highly significant degree, but the effect due to the cream of tartar was the more pronounced.[2] This is shown graphically in Figure 2.

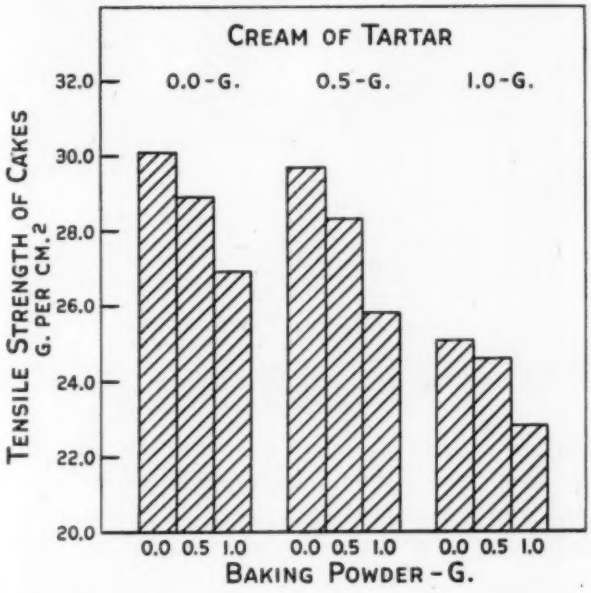

Fig. 2. Effect of three levels of cream of tartar and of baking powder upon the tensile strength of sponge cakes made from spray-dried whole-egg powder.

The average volume of the cakes baked from 175 g of batter varied from 799 cc to 868 cc for the 90 cakes of Series IIa, and from 802 cc to 855 cc for the 45 cakes of Series IIb. The average specific volumes (volume in cc per gram of weight) of cakes from each formula are shown in Table I. The analysis of variance showed that the variation in volume due to cream of tartar was nonsignificant, but that due to the baking powder was highly significant.[3] The cakes baked from the

[2] The F value, or the ratio of the mean square of the variance of the treatment to the mean square for error, was 65.01 for the cream of tartar, and 26.65 for the baking powder. According to Snedecor's table of F values (Snedecor, 1940), the F value necessary for significance at the 5% level is 3.11; for the 1% level, 4.88.

[3] The F value for the mean square for the cream of tartar was 2.36; for the baking powder, 37.35. For values necessary for significance, see footnote 2.

formulas D, G, and I, containing 1.0 g baking powder, were of greatest volume. The effect of the two variables—cream of tartar and baking powder—is shown in Figure 3.

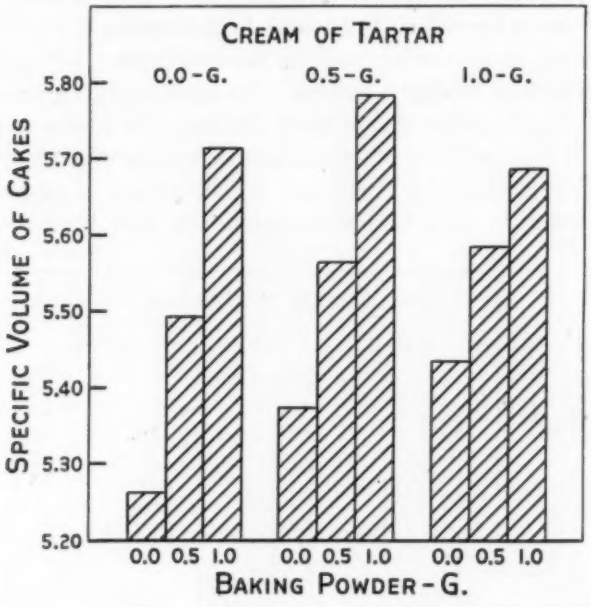

Fig. 3. Effect of three levels of cream of tartar and of baking powder upon the volume of sponge cakes made from spray-dried whole-egg powder. Specific volume is expressed as cc per g of weight.

The coefficient of correlation between the volume and the tensile strength was found to be −0.56, a value which was statistically highly significant, but which was not large enough to indicate that increase in volume is always accompanied by decrease in tensile strength (or increase in tenderness). For example, the cakes baked by formula G were of larger volume than the other cakes, but were not as tender as some of the cakes of smaller volume, and were not so desirable from the standpoint of fineness of grain.

Formula A, in which 1.0 g of cream of tartar and 0.5 g of baking powder were included, consistently gave cakes of fine grain, of good volume (though not the largest volume), and of acceptable tenderness (though not the greatest tenderness). Formula B, in which neither cream of tartar nor baking powder was included, usually produced the least tender and the smallest cakes. Formula I, in which 1.0 g each of cream of tartar and baking powder was used, yielded cakes which were usually slightly more tender and of slightly larger volume than the A cakes. Typical slices of cakes made from formulas A, B, and I are illustrated in Figure 4.

The pH values of the batters of the 45 cakes baked from the nine formulas ranged from 5.50 to 7.65 (see Table I). Batters containing no cream of tartar had average pH values ranging from 7.22 to 7.65; those containing 0.5 g cream of tartar, 6.29 to 6.41; and those containing 1.0 g cream of tartar, 5.50 to 5.64. The coefficient of correlation

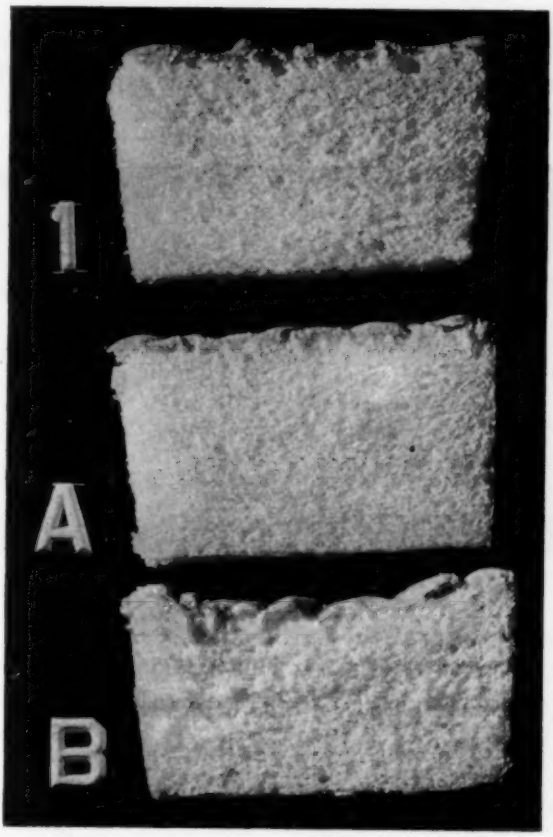

Fig. 4. Typical cakes baked from formulas B, A, and I.

	Cream of tartar	Baking powder
	g	g
Cake B	0.0	0.0
Cake A	1.0	0.5
Cake I	1.0	1.0

between the pH of the batters and the tensile strength of the finished cakes was found to be +0.34, a value significant at the 5% level. However, this low figure would indicate that other factors in addition to the pH of the batter tend to influence the tenderness of the cakes.

The specific gravity and the pH of the batters, and the specific volume and tensile strength of these cakes made from spray-dried

whole-egg powder, compared favorably with corresponding measurements reported by King, Morris, and Whiteman (1936) and King, Whiteman, and Rose (1936) on cakes made from fresh eggs. The specific gravity of the batters and of the cakes also fell within the range advocated by Pyke and Johnson (1940) for sponge cakes baked at altitudes of 0 to 2500 feet.

Typical of the family-size cakes made from the egg powder is the one shown in Figure 5. The formula used was three times the Formula I that was used in the small cakes and was equivalent to a six-egg sponge cake.

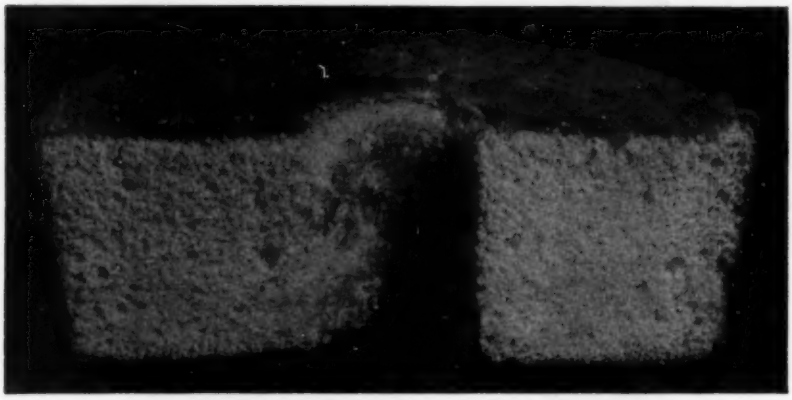

Fig. 5. Typical family-size sponge cake baked from spray-dried whole-egg powder.

The time for beating the larger amount of egg-sugar-water mixture to a satisfactory foam was somewhat longer. It was found advantageous to add the flour gradually, rather than all at once. Baking the cake at 325°F (162.8°C) for 50 minutes gave satisfactory results.

Summary

The leavening property of spray-dried whole-egg powder was tested by using it for making sponge cakes. Acceptable cakes were made both with and without the addition of a secondary leavening agent (baking powder) to the formula.

Temperature was found to be an important factor in the production of a satisfactory egg-sugar foam or meringue from dried eggs. When the initial temperature of the egg-sugar-water mixture was 140°F (60°C), and beating was done on an electric mixer with a wire whip, a foam of suitable lightness for a small-size sponge cake was produced in 3.5 to 7.5 minutes of beating time.

Cream of tartar increased the tenderness of the cakes. The addition of a small amount of baking powder to the formula increased the

volume somewhat and contributed to some extent to the tenderness of the cakes. Hence, a modified sponge cake formula in which some baking powder is included is recommended for use if dried eggs are used for sponge cakes.

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CHEMICAL STATE OF PHOSPHORUS IN STARCH AS INDICATED BY TITRATION CURVES ON ELECTRODIALYZED STARCHES [1]

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Starches from all sources, as ordinarily prepared, contain phosphorus in small but variable amounts. There is always an amount of phosphorus which cannot be removed from the starch by dialysis or extraction with aqueous solvents. The state of chemical combination of this phosphorus appears to vary with the plant source of the starch. Samec (1927) has been chiefly responsible for showing that in certain starches (e.g., potato starch) phosphorus, as phosphoric acid, is in

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ester combination with carbohydrate constituents of the starch, giving to the starch the properties of a colloid electrolyte. That this type of combination of starch with phosphorus may not exist for cereal starches is indicated by the fact (see Samec and Kleman, 1931) that these starches do not show the marked acidic properties, upon electrodialysis, that are characteristic of potato starch. Posternak (1935) showed that the phosphorus of potato starch, after acid hydrolysis, remained in combination with glucose, while the phosphorus of wheat and corn starches, after similar hydrolysis, was found to exist in combination with glycerol. Evans and Briggs (1941) found that methanol extraction of air-dry corn starch removed only about one-third of the total phosphorus while removing 95% of all lipid matter. The phosphorus removed was not identifiable as phospholipid. On the other hand, Schoch (1942) reported that practically all the phosphorus of wheat starch can be extracted with 80% dioxane and believes that most of the phosphorus in wheat starch is in phospholipid combination. Lehrman (1945) attained similar removal of phosphorus from wheat starch by a long-time extraction with methanol.

The present paper reports an attempt to learn something more about the state of the phosphorus in potato, corn, and wheat starches by a study of the form of the titration curves of the exhaustively electrodialyzed starches.

Materials and Methods

Starches. Six potato starches were studied, a commercial potato starch, PVI, and five samples prepared from varieties grown on fertilizer plots by the Division of Soils (University of Minnesota). Of the latter, PI and PV were from Cobbler potatoes grown on check and phosphorus-fertilized plots respectively, and PII, PIII, and PIV were from check plot plantings of Red Warba, Early Ohio, and Triumph varieties, respectively. Samples of the commercial potato starch were also methanol extracted (PVI, DF), and moisture heat-treated (PVI MH) according to the method of Sair and Fetzer (1944) before being electrodialyzed and their titration curves run.

Two samples of corn starch, a commercial product (C) and the same starch after defatting (CDF) by methanol extraction, were studied. These were the same starch samples which were studied by Evans and Briggs (1941).

Two wheat starches, a commercial starch (W) and the same after a methanol extraction (WDF), which was similar to that used in the preparation of corn starch (CDF), were employed in the study.

Electrodialysis of the Starches. Titration curves on colloid electrolytes can be dependable only when all diffusible electrolytes and

counter ions (other than H^+ or OH^- ions) have been removed from the high molecular weight (or colloid) components. Often simple dialysis of the aqueous solution or suspension of the colloid against distilled water followed by an extended period of electrodialysis is able to accomplish this process without causing any other changes in the physical or chemical properties of the colloid. This simple procedure is not successful with starch for the following reasons. The phosphorus present in starch (e.g., potato starch), which cannot be removed by simple dialysis or extended washing of the starch granules with distilled water, may exist in two forms, as insoluble calcium phosphate and as the mono ester of phosphoric acid—calcium salt with the carbohydrate. In order to render the calcium phosphate soluble and to mobilize the calcium of the ester salt it is necessary to conduct the dialysis (and electrodialysis) at a low pH. Under this condition of low pH there is a marked danger, due to the long time usually required for electrodialysis, that some hydrolysis of the polysaccharide will occur. The following procedure has been found effective in removing any insoluble calcium phosphate (not esterified), together with practically all counter ions (including calcium) combined with the esterified phosphorus, from ungelatinized starch without causing any measurable hydrolysis. The starch sample (granules) is mixed, in a tall cylinder, with two to three times its volume of a 0.1 *N* hydrochloric acid solution saturated with sodium chloride. This is allowed to stand with occasional shaking at room temperature for an hour, after which the granules are allowed to settle and the supernatant solution is removed and replaced by a similar volume of 0.1 *N* acid solution half saturated with sodium chloride which is allowed to remain in contact with the granules for one hour. The procedure is repeated with a solution of the 0.1 *N* acid one-quarter saturated with sodium chloride and finally with repeated changes of distilled water until, after standing for a few minutes, the test for chloride is quite faint in the contact water. In this process the acid reaction serves to mobilize the calcium of the phosphate and ester-phosphate. The sodium chloride, by base exchange action, causes the replacement of calcium ions in the starch granule by sodium, and the exhaustive washing serves to remove most of the excess salt and the acids.

The washed starch granules are then packed into the thin center compartment of an efficient three-compartment electrodialysis apparatus and electrodialyzed. In this apparatus, the middle compartment was only one cm in thickness and a D.C. voltage drop of 100–150 volts per cm could be maintained across this starch diaphragm until most of the diffusible ions were removed. The entire operation may be accomplished in an elapsed time of 12 to 15 hours. During

the electrodialysis the temperature of the starch was never allowed to go above 30°C, the dialyzer being kept cool by a glass cooling coil placed in the center compartment. The calcium contents of the electrodialyzed starches given in Table I indicate that an efficient removal of this cation was accomplished, and the alkali lability values on the starches prior to and after the treatment indicate that no detectable hydrolytic degradation occurred.

Analyses of the Starches. The calcium contents of the starches were determined in duplicate by the method of Kramer and Tisdall (1921). Samples of starch varying from 5 to 15 g of dry starch were dried in a platinum dish at 105°C for 12 hours, and the dry weight determined. The dried samples were ashed by placing in a cold muffle and heating slowly to 400°C until charred (frothing was prevented in this manner), then heating at 600°C for 6 hours. The ash was fused with sodium carbonate, dissolved in 6 *N* hydrochloric acid, and the solution transferred to a 50 ml conical centrifuge tube. Five ml of saturated ammonium oxalate and 2 drops of bromcresol purple were added and the pH adjusted with ammonium hydroxide to about pH 6.2. The sample was allowed to stand for 6 hours, centrifuged, washed with 2% ammonium hydroxide, centrifuged and drained, and 10 ml of 3 *N* sulfuric acid added. The sample was then titrated with 0.01 *N* potassium permanganate and the calcium content calculated. Agreement between duplicates was usually within 3%. Average values are given in Table I.

Phosphorus contents of the starches were determined in triplicate by a modification of the colorimetric method of Fiske and Subbarow (1925) in which samples of dry starch weighing 0.1 g to 0.3 g (1.0 g samples in case of the corn starches) were wet-ashed with sulfuric and nitric acids and the color which developed upon the addition of ammonium molybdate and 1-amino-2 naphthol-4-sulfonic acid was estimated in a Coleman spectrophotometer at wave length 725 mμ. Phosphorus content was obtained by comparison with a standard curve made with the same procedure and instrument. The triplicate determinations usually agreed within 10%. Average values are given in Table I.

Alkali lability values on the starches were determined by the method of Schoch (1940).

pH Measurements on the Starch Pastes. One gram (dry weight basis) samples of the electrodialyzed starch granules were placed in 50 ml Erlenmeyer flasks and 30 ml of carbon dioxide free distilled water containing appropriate increments of carbon dioxide free sodium hydroxide were added. Each sample was gelatinized by placing the flask, while being rotated to maintain the starch granules in suspen-

TABLE I

CALCIUM, PHOSPHORUS CONTENTS, ALKALI LABILITY VALUES OF STARCHES BEFORE AND AFTER BASE-EXCHANGE-ELECTRODIALYSIS AND ACIDIC CAPACITIES OF THE ELECTRODIALYZED STARCHES

Starch	Calcium (per g starch)				Phosphorus (per g starch)				Alkali lability		Acidic capacity (per g starch)			
	Before elect.		After elect.		Before elect.		After elect.		Value		Below pH 5		Between pH 5 and pH 8.7	
	mg	meq	mg	meq	mg	meq	mg	meq	Before elect.	After elect.	meq	A[1]	meq	B[1]
Potato PI	0.14	0.007	0.006	0.0003	0.430	0.014	0.413	0.013[3]	4.3	5.2	0.012[6]	.95	0.021[5]	1.61
Potato PII	.11	.005	.014	.0007	.515	.017	.492	.015[9]	—	—	.014[4]	.91	.024[3]	1.53
Potato PIII	.32	.016	.019	.0009	.675	.022	.640	.020[7]	7.6	6.4	.019[5]	.94	.031[2]	1.51
Potato PIV	—	—	.012	.0006	.520	.017	.502	.016[2]	—	—	.015[1]	.93	.024[3]	1 49
Potato PV	—	—	016	.0008	.510	.016	.415	.013[4]	—	—	.012[1]	.90	.021[5]	1.60
Potato PVI	.49	.024	.011	.0005	.693	.022	.650	.021[0]	6.1	6.3	.019[6]	.93	.027[6]	1.31
Potato PVI DF	—	—	—	—	—	—	.612	.019[7]	—	—	.018[6]	.94	.024[5]	1.24
Potato PVI MH	—	—	—	—	—	—	.460	.014[9]	—	—	.013[8]	.94	.018[2]	1.22
Corn C	.18	.009	.012	.0006	.154	.005	.137	.004[4]	13.2	12.9	0	—	.022[0]	5.50
Corn CDF	.13	.006	.001	—	.095	.003	.082	.002[6]	13.2	12.7	0	—	.002[4]	0.92
Wheat W	.31	.015	.011	.0005	.570	.018	.550	.017[8]	—	—	0	—	.006[0]	3.00
Wheat WDF	.22	.011	.008	.0004	.508	.016	.402	.013[0]	11.7	10.2	0	—	.001[6]	0.08

[1] $A = \frac{\text{meq acid titrated below pH 5.0}}{\text{meq phosphorus}}$ [2] $B = \frac{\text{meq acid titrated between pH 5.0 and pH 8.7}}{\text{meq phosphorus}}$

sion, in a boiling water bath for 10 minutes. The pastes so obtained were cooled to room temperature and their pH values determined with a glass electrode assembly employing a L. and N. vacuum tube voltmeter, student potentiometer, and type R galvanometer. With this apparatus, pH values accurate to 0.01 pH units were attained. Titration curves obtained by plotting the observed pH values against the equivalents of sodium hydroxide added to the corresponding samples were smooth and accurately repeatable.

Results and Discussion

Titration Curves for Electrodialyzed Potato Starches. The titration curves for the eight samples of potato starch, three of which are shown in Figure 1, all exhibit the two distinct buffer ranges characteristic of the first and second hydrogens of phosphoric acid. Titration of the first hydrogen is complete at about pH 5.0 and of the second hydrogen at about pH 8.75. The amount of alkali necessary to complete the neutralization of the first hydrogen is consistently from 5 to 10% less than the amount calculated as necessary from the phosphorus content of the electrodialyzed starch (A in Table I). This may be taken to indicate that the base exchange-electrodialysis process failed to remove a small amount of counter-ion. Analysis for calcium shows that not all of the retained counter-ion was calcium and indicates that a small amount of sodium must also have been retained. Dissociation constant calculations for the most acid hydrogen in the potato starches using the Van Slyke (1922) equation, $Ka = \frac{[H]([B] + [H])}{[S] - [B] - [H]}$, yielded values for pKa of 2.7 for PVI to 3.1 for PI and PV which are considerably higher than that accepted for the first hydrogen of orthophosphoric acid (pKa = 2.03).

In Figure 1 is given the titration curve for an aqueous solution of orthophosphoric acid containing the same concentration of phosphorus as the potato sample PVI titrated. The shift of the titration curve, corresponding to the first hydrogen of the starch phosphoric acid, toward the alkaline range as compared with that of the free phosphoric acid may be due to an actual decrease in the dissociation tendency of this acidic hydrogen when the phosphoric acid is esterified with the starch. However, the dissociation tendencies of both the first and second hydrogens of phosphoric acid, when esterified with glucose, are reported to have increased as a result of the ester formation. The pK_1 value for glucose-1-phosphate has been reported by Cori, Colowick, and Cori (1937) to be 1.11, while that for glucose-6-phosphate was found to be 0.97 by Meyerhof and Lohmann (1927). It seems that a more likely explanation for the decrease in pK_1, observed

in this case, is that it is due to a Donnan equilibrium effect. The electrode measures the hydrogen ion activity of the solution outside but in Donnan equilibrium with the region inside the swollen starch granules. The deviation of an observed pK value from its true value

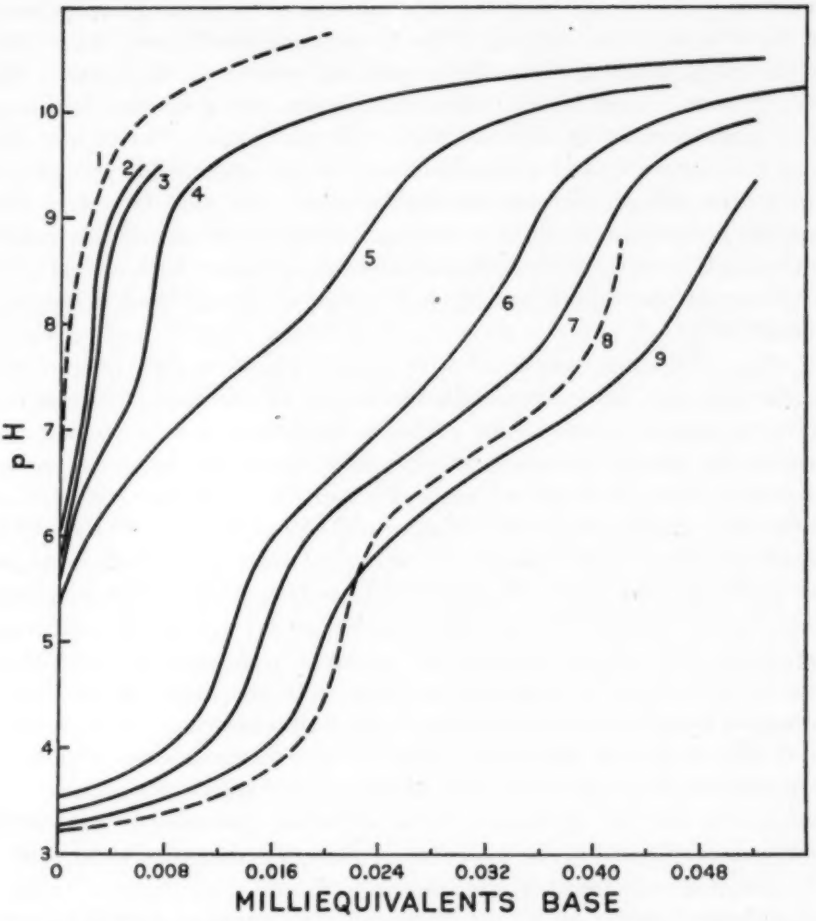

Fig. 1. Titration curves on electrodialyzed starches. Curve 2—Methyl alcohol-extracted wheat starch, WDF; Curve 3—Methyl alcohol-extracted corn starch, CDF; Curve 4—Commercial wheat starch, W; Curve 5—Commercial corn starch, C; Curve 6—Cobbler potato starch, PI; Curve 7—Red Warba potato starch, PII; Curve 9—Commercial potato starch, PVI; Curve 1—H_2O; Curve 8—Orthophosphoric acid solution of same phosphorus content as that of potato starch PVI. Volume of the solutions = 30 ml. Starch solutions contained 1 g each of starch.

would be in the direction observed and would be greatest for starches containing the lowest amounts of phosphorus. No dependable value can, therefore, be assigned for the pK_1 from our titration data, although it is probable that the starch-phosphoric acid is as strong an acid as orthophosphoric acid itself.

The leg of the titration curve lying between pH 5.0 and 8.75, while yielding an over-all value of pK_2 (using the Henderson Hasselbalch equation) which agrees well with the second hydrogen of orthophosphoric acid (observed $pK_2 = 6.75$ for starch PVI, while accepted value for orthophosphoric acid is 6.8), requires more alkali to titrate it than would be expected from the amount of total phosphorus found in the starch (B in Table I). The latter accounts for only 62 to 76% of the alkali needed, this varying with the source of the starch. Obviously some group besides the second hydrogen of starch phosphoric acid ester is reacting with alkali in this pH range. Because of this and the superimposed Donnan effect, an accurate value for pK_2 of the starch-phosphoric acid cannot be given. It may be safely concluded, however, that the first and second hydrogens of potato starch-phosphoric acid ester have dissociation tendencies which are as great as those of the corresponding hydrogens of unesterified orthophosphoric acid.

The phosphorus esterified with potato starch is not constant per gram of starch, but varies with the source of the starch. From the data presented here it seems probable that there is less variation in bound phosphorus between starches of potatoes of the same variety grown on different fertilizer plots (PI and PV) than there is between different varieties grown on the same soil (PI, PII, PIII, PIV). Only small amounts of phosphorus are removed from any of the starches by the base exchange and electrodialysis treatment. Hot methanol extraction followed by the base exchange-electrodialysis treatment removed only a little more of the potato starch phosphorus. Moisture-heat treatment followed by base exchange and electrodialysis removed about a third of the potato starch phosphorus. It is probable that the amount of phosphorus mobilized in all these cases represents a corresponding degree of hydrolysis of the starch-phosphoric acid linkage in the potato starch. The esterified phosphorus content of potato starch can thus be expected to be a function to some extent of the previous treatment of the starch.

Titration Curves for Cereal Starches. The titration curves for corn and wheat starches, both before and after hot methanol extraction, show distinct differences from those of the potato starches. In the first place there is no evidence of a buffer range corresponding to the first hydrogen of phosphoric acid. Wheat starches, before and after methanol extraction, show a small degree of buffer capacity in the pH region in which the second hydrogen of potato starch phosphoric acid ester is titrated, but this is much less than would be expected from the phosphorus contents of these wheat starches and is not greatly changed by the methanol extraction of the starch. Corn starch, undefatted,

shows a marked buffer capacity in this region of pH which is considerably greater than could be accounted for by its phosphorus content. This buffer capacity of corn starch is almost completely removed when the starch is defatted. Evans and Briggs (1941) found that the free fatty acid content of this starch was 0.0052 g per gram of starch, these acids having an average molecular weight of 275. This is equal to 0.02 milliequivalents of fatty acid per gram of starch. The difference observed in the buffer capacity of corn starch (in this pH region) before and after defatting was .019 milliequivalents. Obviously the adsorbed but unesterified fatty acids of the corn starch are responsible for most of this buffer capacity of the undefatted corn starch. It is surprising that fatty acids should be titratable in this pH range (pKa = 7.5) since most such acids in aqueous solution act as considerably stronger acids (pKa values approximately 5). The strongly adsorbed state of the free (unesterified) fatty acids in starch apparently causes a shift in their dissociation tendency toward that of a weaker acid.

The very small residual buffer capacity of defatted corn starch may well be due to a residual amount of fatty acid not removed by the methanol extraction. The low buffer capacity of the wheat starches in this same pH region may also be due to small amounts of unesterified but strongly adsorbed fatty acids in the starch. No such adsorbed fatty acids have been found in potato starch, yet we find a considerable buffer capacity for potato starch in this same pH range which cannot be accounted for by the phosphoric acid present. Also, exhaustive methanol extraction of potato starch fails to reduce this extra buffer capacity to any appreciable degree. There must, therefore, remain the possibility that there is some material (or group) present in potato starch, and possibly in wheat and corn starches, which is not removable by methanol extraction or by exhaustive electrodialysis and which shows a buffer capacity in the pH region between 5.0 and 8.7. This might be silicic acid or some protein constituent. No definite identity can be conferred on it at present.

If any of the phosphorus present in the cereal starches were combined as simple ester with the starch, either as mono or di ester, it should be detectable in the titration curve of the electrodialyzed starch as a buffer zone corresponding to the first hydrogen of orthophosphoric acid (at the appropriate dilution corresponding to the amount of phosphorus present in such form in the starch). Even very small amounts should be detectable. The curves for the cereal starches show no indication that any such phosphorus exists in these starches. This does not necessarily mean that none of the phosphoric acid in corn or wheat starch is in ester combination with the starch. It does

mean, however, that, if such an ester exists, either it is the tri ester or the phosphoric acid is in mono ester combination with starch and has a strongly alkaline group esterified through a second group. The zwitter ion which would result would act similarly to lecithin where electrodialysis can take the pH of its solution only to the isoelectric point of the zwitter ion. The fact that a large part of the phosphorus of wheat starch is of a phospholipid nature could emphasize the probability that, if any part were in combination directly with starch, it too might be similarly combined with a basic group as in the phospholipid.

Above a pH of 9.0 *all* the starches show a very strong buffer action toward added alkali, the extent of which is apparently independent of the source of the starch. In this alkaline region there is either a strong tendency to adsorb alkali, or, more likely, one or more of the hydroxyl groups on the sugar units of the starch molecule evince their acidic nature by reacting as acids to form salts with the added alkali.

Summary

Titration curves on exhaustively electrodialyzed potato starches show buffer ranges which correspond well with those of the first and second hydrogens of orthophosphoric acid. That corresponding to the first hydrogen agrees well with that expected from the total phosphorus content of the starch. That corresponding to the second hydrogen is from one-fourth to one-third greater than can be accounted for by the phosphorus present in the starch.

Titration curves for exhaustively electrodialyzed wheat and corn starches show no buffer capacity in the pH range in which the first hydrogen of the potato starch acid is titratable. Both show buffer capacity in the pH region in which the second hydrogen of potato starch acid is titratable, but their buffer capacities in this region are not related to the phosphorus contents of the starches. With corn starch this buffer capacity is correlated with the unesterified adsorbed fatty acid content of the starch. It is postulated that the slight buffer capacity of wheat starch in this pH region may be due to the presence of free fatty acids or to groups (or material) similar in nature to the unidentified material in potato starch which is responsible for the excess buffer capacity of this starch over that accountable for on the basis of its phosphorus content.

None (or no detectable amount) of the phosphorus present in cereal starches exists in the mono-orthophosphoric acid ester form which is characteristic of all the phosphorus of electrodialyzed potato starch.

Above pH 9.0 all starches show an equal tendency to neutralize alkali, and this occurs to a relatively much greater degree than is characteristic of the buffer capacity of any starch at lower pH ranges. This probably involves the ionization of hydrogen in the hydroxyl groups of the sugar monomers of the starch.

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PREPARATION AND NITROGEN CONTENT OF SOYBEAN PROTEIN

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The term "soybean protein" usually refers to the curd which is precipitated from an aqueous or alkaline extract of soybean meal by addition of acid. It has become a commercial product of considerable importance. The solution remaining after removal of the curd is known as whey and has no value at present, although it is well known to contain additional protein. Since water alone extracts at least 90% of the nitrogenous material from soybean meal and alkali practically all of the nitrogen, the curd and whey contain nearly all the protein of the soybean, although such high yields are not attained in commercial operation.

The separation of soybean proteins was first studied by Meissl and Böcker (1883), and later more extensively by Osborne and Campbell (1898) who named the principal protein "glycinin." Osborne's glycinin is defined as "the salt solution soluble globulin which separates after dialysis"; however, it has not been identified as a single protein. Thus, the question is raised as to the purity of the "soybean protein" prepared by commercial methods in comparison with Osborne's "glycinin."

In the absence of crystallinity or distinctive physical properties, the nitrogen content is the most convenient method of determining the purity of a protein. The variations in nitrogen content of different preparations of soybean protein from the same source material might be due to nonprotein impurities or to fractionation into proteins of different composition. We shall present evidence that the former appears to be the more important factor. Jones and Csonka (1932) and also Ryndin, Morozov, and Salchinkin (1936) separated glycinin into fractions having different physical properties, while Csonka and Jones (1933) showed that the nitrogen content and amino acid composition of glycinin vary with the variety of soybean.

The following methods have been used in separating soybean protein and glycinin from the nonprotein parts of soybean meal and from the minor proteins (albumin and proteose):

A. Extraction with water and precipitation with acid.
B. Extraction with dilute alkali and precipitation with acid.

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C. Extraction with a salt solution (usually 1–2N NaCl) and precipitation by dialysis or dilution.

D. Extraction with water or salt solution, precipitation by saturating with ammonium sulfate, redispersing in water, and precipitation by dialysis.

Methods B and C may be repeated one or more times to obtain a purer product. Methods A and B are essentially the procedures used industrially; most chemists have followed Osborne and Campbell in preferring C and D. Method B gives the largest yield, since it includes the less soluble part of the protein. The insoluble protein fraction in the meal residue can be decreased by fine grinding of the meal (Smith, Circle, and Brother, 1938). It is possible that it does not actually represent a distinct protein, but protein retained in intact cells as suggested by Woodruff, Chambers, and Klaas (1938).

Table I shows the nitrogen content of various protein preparations reported in the literature. The methods of preparation are indicated by the letters used above, successive letters showing purification steps. An asterisk indicates that the final precipitate was dehydrated with alcohol.

TABLE I

NITROGEN CONTENT OF GLYCININ AND SOYBEAN PROTEIN FROM THE LITERATURE

Authors	Variety of bean	Method of preparation	N [1]	Yield [2]
			%	%
Osborne and Campbell (1898)	"Yellow soybean"	D *	17.14	7.7 [3]
		DCC *	17.72	
	"Kiyusuki diadzu"	D *	17.45	6.1 [3]
		DCC *	17.48	
Jones and Moeller (1928)	"Yellow soybean"	"Method of Osborne and Campbell"	17.04	—
Csonka and Jones (1933)	Illini	C *	17.74	—
	Dunfield	C *	17.37	—
	Manchu	C *	17.28	—
	Peking	C *	17.19	—
	Dixie	C *	16.60	—
Hartman and Cheng (1936)	—	CC *	17.61	10.0
Ryndin (1936)	—	CC *	17.35	5.4
Woodruff, Chambers, and Klaas (1938)	Illini (commercial flakes)	A *	16.40	25.3
		C *	16.44	10.4
Smith and Circle (1939) [5]	Illini	A	15.68	29.4
Pucher and Nolan; see Vickery (1945)	Illini	CC [4]	16.93	—

[1] On ash-free dry basis.
[2] On weight of oil-free meal.
[3] Total of several fractions.
[4] Dried with acetone.
[5] Not specially prepared for high purity.
* Indicates dehydration by alcohol.

A. Water extraction-acid precipitation.
C. Salt extraction-precipitation by dialysis.
D. Water or salt extraction-ammonium sulfate precipitation.

The range of variation in the nitrogen content seems quite surprising. Jones (1931) showed that the nitrogen content of gliadin and glutenin prepared from various varieties of wheat by many different workers was fairly constant, the maximum variation being 0.68%, and asserted that the same was true of other vegetable proteins. The data on glycinin and soybean curd protein in Table I differ more widely than this, even if we confine ourselves to one paper (Csonka and Jones) or to one variety (Illini).

The nitrogen content of soybean protein is of importance also because of the use of factors in food and nutrition studies to determine the protein content of soybean products. The conventional factor of 6.25 is still in use for this purpose. Jones (1931), while pointing out the fundamental weaknesses of this method of determining protein, nevertheless recommended a factor based on the nitrogen content of glycinin, taken as 17.5%. There appears to be little value in adopting a new factor if the nitrogen content of glycinin is as uncertain as Table I indicates.

In addition to comparing the purity of soybean protein prepared by commercial procedures with Osborne's glycinin, the object of the present work was to determine the effect of various factors on the nitrogen content of soybean protein, and especially the relative importance of the source of material, and of the method of preparation in accounting for the variations. We have found that the nitrogen contents obtained by different methods of purification tend to reach an upper limit that is not very different for different varieties and crop years. Most of the variation in our preparations appears to be due to a nonprotein impurity.

Methods and Materials

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, using a mercury catalyst and a one-hour digestion. Chibnall, Rees, and Williams (1943) have shown that a longer period of digestion is essential for egg albumin, and raised the question as to the accuracy of nitrogen determination on other proteins when a short digestion period is used. To check the feasibility of the method for isolated soybean protein, a series of tests was run using 1-, 3-, and 7-hour digestion periods with mercury and copper sulfate catalysts. The results are shown in Table II, where each value is the average of two or more determinations.

From these results, and a Dumas nitrogen determination on the same sample which gave a value of 15.83%, it was concluded that, for an isolated soybean protein, a digestion period of one hour was sufficient. Ash was determined in platinum crucibles at 650°C. Por-

TABLE II

THE EFFECT OF DIGESTION TIME ON THE KJELDAHL NITROGEN DETERMINATION FOR ISOLATED SOYBEAN PROTEIN

Date	Catalyst	Digestion period and percent nitrogen		
		1 hour	*3 hour*	*7 hour*
2–2–44	Hg	15.71	—	—
6–8–44	Hg	15.62	—	—
6–27–45	Hg	—	15.74	15.78
7–2–45	Hg	15.87	—	15.80
6–27–45	$CuSO_4$	—	15.75	—
7–2–45	$CuSO_4$	—	—	15.83
Average	—	15.73	15.75	15.80
Dumas nitrogen	—	15.83	—	—

celain crucibles gave irregular and much higher results. Moisture was determined by drying in a vacuum oven at 105°C for 5 hours. All determinations were made at about equilibrium moisture content, and the nitrogen values corrected for ash and moisture. This is preferable to attempting to analyze the very hygroscopic dried protein.

With the exception of Csonka and Jones (1933), who used ethyl ether, all the authors quoted in Table I used light hydrocarbons (petroleum ether, benzine) to extract the oil from their soybean meal. It has been found in this laboratory (Beckel and Smith, 1944; Belter, Beckel, and Smith, 1944) that soybean meal from which the oil has been extracted with ethanol gives a much lighter-colored protein than meal extracted with other solvents, suggesting that some impurity has been removed. In this study, soybean protein has been prepared from meals extracted with ethanol and with a commercial petroleum ether (Skellysolve F), and the nitrogen contents have been compared. The varieties and crop years used were: Illini, 1941, 1942, 1943; Peking, 1941; Mandarin, 1942; Lincoln, 1943; and Willomi (81044), 1943.

After cracking, dehulling, and flaking, the beans were extracted in a modified Soxhlet-type extractor in which the solvent entered the extractor through a condenser; thus the flakes were kept substantially at room temperature. Soybean protein was also prepared from flakes which were ethanol-extracted on a pilot-plant scale at about 60°C, and from commercial solvent-extracted "brew" flakes having a high water-soluble protein content. For laboratory protein preparations, the flakes were ground in a hammer mill. Results are also given on some proteins prepared in a pilot plant (Belter, Beckel, and Smith, 1944).

Protein Preparations. Table III shows the methods of preparation and nitrogen contents of the protein prepared in this laboratory. The

TABLE III

LABORATORY SOYBEAN PROTEIN PREPARATIONS

No.	Variety and year	Solvent and method	N [1]	Yield [2]
			%	%
1	Illini 1941	Pet. ether AC	16.73	—
2	Illini 1942	Pet. ether C	16.37	—
3	Illini 1942	Pet. ether CC	16.68	23
4	Illini 1942	Pet. ether CCC	16.51	—
5	Illini 1942	Pet. ether D	16.48	19
6	Illini 1942	Pet. ether AB	15.91	—
7	Illini 1942	Pet. ether ABBB	15.98	26
8	Illini 1942	Pet. ether ABBBBB	15.99	—
9	Illini 1942 [3]	Pet. ether A pH 5.4	16.18	32
10	Filtrate from No. 9	pH 4.0	14.95	5
11	Illini 1942	Ethanol B [4]	16.16	—
12	Illini 1942	Ethanol BB	16.27	37
13	Illini 1942	Ethanol BBB	16.32	—
13a	Illini 1942	Ethanol CC	16.67	Low
14	Illini 1943	Ethanol CC *	16.62	—
15	Illini 1943	Ethanol A *	16.47	—
16	Illini 1943	Ethanol ABB *	16.36	—
17	Illini 1943	Ethanol B *	16.00	34
18	Illini 1943	Ethanol BBB *	16.13	—
19	Peking 1941 [3]	Pet. ether AB	15.30	39
20	Peking 1941 [3]	Pet. ether ABB	15.31	—
21	Peking 1941	Pet. ether A	15.58	—
22	Peking 1941	Pet. ether ABB	15.78	36
23	Peking 1941	Ethanol A	16.08	—
24	Peking 1941	Ethanol ABB	16.48	32
25	Lincoln 1943	Ethanol B	15.41	—
26	Lincoln 1943	Ethanol BBB	16.26	38
27	Lincoln 1943	Ethanol BBBB	16.20	—
28	Willomi 1943	Ethanol AB	16.78	—
29	Willomi 1943	Ethanol ABB	16.90	24
30	Willomi 1943	Ethanol B	15.43	—
31	Willomi 1943	Ethanol BB	15.83	43
32	Willomi 1943	Ethanol BBB	16.19	—
33	Mandarin 1942	Pet. ether A	15.86	—
34	Mandarin 1942	Pet. ether A *	16.17	—
35	Mandarin 1942	Pet. ether ABB	16.05	—
36	Mandarin 1942	Pet. ether ABB *	16.32	—
37	Mandarin 1942	Ethanol A *	16.59	20
38	Mandarin 1942	Ethanol BB	16.70	—
39	Mandarin 1942	Ethanol BBB	16.88	38
40	Mandarin 1942	Not extracted AB *	16.21	—

[1] On ash-free dry basis.
[2] Grams of air-dry protein per 100 g of air-dry, fat-free meal.
[3] Including hulls—all others dehulled.
[4] Extracted in pilot plant at 60°C.
* Indicates dehydration by alcohol.

A. Water extraction—acid precipitation.
B. Alkali extraction—acid precipitation.
C. Salt extraction—precipitation by dialysis.
D. Water extraction—ammonium sulfate precipitation.

methods of preparation are designated by the same symbols as in Table I. In Method B, 0.1% sodium hydroxide was usually used in the original extraction, while in the reprecipitation steps, only enough alkali was used to disperse the protein (pH, about 9). Precipitation in Methods A and B was at pH 4.1–4.5, usually with sulfuric acid. In Method C, 6% (approximately 1N) sodium chloride was the dispersing agent; following dialysis, the pH was adjusted when necessary

to the above range. In succeeding steps, dilution with several volumes of water was used in place of dialysis. Method D was carried out by water extraction (Osborne and Campbell used salt extraction). Dehydration by alcohol is indicated by an asterisk. In other cases, the glycinin was dried at 65°C in a forced-draft oven. Yields are given only where at least two extractions were made on the meal, in order to obtain maximum yield.

The separation of the first extract from the insoluble residue has always been a difficulty in both laboratory and large-scale production of protein. In these experiments, this was done by centrifuging at about 2000 *g*. Especially with water extraction, the boundary between the extract and the residue is not sharp. This is a possible cause of irregularities in the nitrogen content. Sample 13A, Table III, was given a special filtration treatment with macerated filter paper. After the first salt dispersion, it was filtered twice, and after the second dispersion, it was filtered three times.

The pilot-plant protein preparations are given in Table IV. Unground flakes were used, "commercial" and "ethanol" referring to commercial hydrocarbon-extracted flakes, and pilot-plant ethanol-extracted flakes, respectively. Tap water was used for the alkaline extractions, and distilled water for No. 41. Sulfuric acid was the precipitating agent, except for No. 46 which was precipitated with sulfur dioxide. Sodium bisulfite was used for its bleaching and preservative action.

Discussion

Using the highest nitrogen content obtained for each variety in Table III, we find a variation from 16.26% (Lincoln) to 16.90% (Willomi). This range is no larger than that found for wheat proteins (Jones, 1931), and is in contrast to the large variations shown in Table I. We are unable to explain why no glycinin prepared in this laboratory has ever given as high values of nitrogen as those of Osborne and Campbell (1898), Csonka and Jones (1933), or Hartman and Cheng (1936). However, values in the range of 16.90 to 17.00% nitrogen for samples of protein prepared by very careful work of Dr. G. W. Pucher and Mr. L. S. Nolan in H. B. Vickery's laboratory (Vickery, 1945) are consistent with our results.

More important than variations due to variety and crop year are the variations caused by the method of preparation. These variations are not due to fractionation of the protein, but rather to varying degrees of removal of a nonprotein impurity. This conclusion is based on the following facts:

(1) Some of the preparations of highest nitrogen content which were made from ethanol-extracted meal, as Nos. 11–13, 23–24, and

38–39, were obtained in sufficiently large yields to indicate that they include practically all of the protein. This appears to be the most effective way of removing the impurity.

(2) Dehydrating the wet curd with ethanol also raises the nitrogen content, although not to the same level as ethanol extraction of the meal (compare Nos. 33–37).

(3) Extracting the dried protein with hot ethanol in a Soxhlet extractor raised the nitrogen content in the case of a pilot-plant preparation from commercial flakes (Nos. 41–42), but did not do so for a similar preparation from ethanol-extracted flakes (Nos. 44–45). In the former case, the ethanol extract from 100 g of protein yielded about 4 g of a brown syruplike material; in the latter, less than 0.1 g was obtained.

(4) Methods C and D, which involve a reduced yield and hence considerable fractionation, do not give a much higher nitrogen content than ethanol extraction followed by Method A or B (compare Nos. 2–5, 11–13, 14–18).

The nature of the impurity is still in doubt; it appears to be a complex mixture. The "syrup" mentioned above contains 1.30% nitrogen and 0.79% phosphorus. Acid hydrolysis of the syrup yielded phosphoric acid, fatty acids, and choline (precipitated as the reineckate), indicating the presence of lecithin. Carbohydrates are also present (Molisch reaction). The syrup is largely soluble in most organic solvents and partly so in water. Phosphatides probably act as dispersing agents. The purifying effect of Method C is in harmony with the fact that salts precipitate dispersed phosphatides.

Most of our preparations were made from dehulled beans. Preparations 19–22 show that the inclusion of the hulls adds a further impurity to the protein, as is also shown by its darker color. Soybean hulls contain about 10% of water-soluble material, and the water extract is dark brown.

Preparations 9 and 10 show another method of obtaining a purified protein. A water extract of a petroleum ether-extracted meal, including the hulls, was precipitated at pH 5.4 to give protein having a nitrogen content of 16.18%. The "whey" from this precipitate was adjusted to pH 4 and gave a further small precipitate with a nitrogen content of only 14.95. Evidently, the bulk of the impurity is precipitated at the lower pH. This method offers some difficulty in practice because of the poor coagulation and settling of the precipitate, but it has the advantage of being applicable to present commercial soybean flakes.

Methods C and D (preparations 2 and 5) are about equal to alcohol extraction followed by Method A (preparation 15) in purifying effect, but the latter is more practical for large-scale production. More than

two precipitations are of little or no advantage whether Method B or C is used. Method A would be expected to be better than B for the first extraction, but this is not always the case; Method B not only gives the larger yield but sometimes the higher nitrogen content as well.

The four pilot-plant preparations shown in Table IV illustrate the variations in nitrogen content of glycinin which is encountered in large-scale production. The use of flakes rather than ground meal, washing by settling and decantation, and the considerable time required may be factors in this variation. It is rather surprising that a pilot-plant protein, No. 46, has the highest nitrogen content (17.02%) we have encountered. Preparation 47 represents an attempt to duplicate pilot-plant conditions in the laboratory, including the use of unground hot ethanol-extracted flakes and the addition of sodium sulfite. The result agrees with preparation 44 rather than 46.

TABLE IV

PILOT PLANT-SOYBEAN PROTEIN PREPARATIONS

No.	Flakes	Dispersing agent	N [1]
			%
41	Commercial	H_2O	15.28
42	(No. 41 after ethanol extraction)		15.70
43	Commercial	NaOH	16.32
44	Ethanol	$NaOH+Na_2SO_3$	16.50
45	(No. 44 after ethanol extraction)		16.38
46	Ethanol [2]	$NaOH+Na_2SO_3$	17.02
47	Ethanol [3]	$NaOH+Na_2SO_3$	16.65

[1] On ash-free dry basis. [2] Illini soybeans—1942 crop. [3] Prepared in laboratory—see text.

Preparation 40 was made by the unusual method of extracting protein from unextracted (full-fat) soybean flour and removing the oil later. In this process, it is found that the oil disperses and precipitates with the protein, forming a milklike emulsion and showing little or no tendency to separate, even on reprecipitation. By dehydrating the wet curd with ethanol, nearly all of the oil was removed, but the protein was further purified by washing with petroleum ether and extracting with ethanol in a Soxhlet extractor. The nitrogen content compares favorably with other preparations, but in view of the difficulty in the removal of the oil and the ethanol, this method probably is not practical.

Summary

Soybean protein has been prepared by various methods from several varieties of soybeans, and the nitrogen content has been determined. The variations between varieties are small compared to those due to method of preparation, the highest value obtained for each variety ranging from 16.26% (Lincoln) to 16.90% (Willomi). In

agreement with other recent workers, we have not been able to obtain a value as high as the usually accepted 17.5%.

The variation in nitrogen content of glycinin preparations from a single source appears to be due to an impurity rather than to fractionation. This impurity is best eliminated by using ethanol as the solvent in extracting the oil.

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STALING STUDIES ON BREADS CONTAINING WAXY MAIZE STARCH [1]

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Researches on the complex phenomena which occur during the staling of bread have associated the decrease in compressibility of the crumb with changes in the starch. The hardening of the crumb is accompanied by a decrease in crumb translucency, in the swelling power in water, in the viscosity of its aqueous dispersions, and by a reduction in the amount of water-extractable polysaccharides. The literature on this subject has been reviewed by Katz (1928), Alsberg (1936), Cathcart (1940), and Radley (1943). As a result of his extensive investigations Katz attributed the staling of bread crumb to a "retrogradation" of the starch, a term which has been loosely used in the literature to denote colloidal changes which occur in starch pastes on standing whereby the starch becomes less soluble. His X-ray studies (Katz, 1930, 1934) indicated that the insolubilization of the starch in a paste involves a transition from the amorphous to the crystalline state and that a similar change occurs when bread stales. Alsberg (1936) has pointed out that the retrogradation theory is not in accord with either the rapidity or the reversibility of the staling process. Even concentrated starch pastes retrograde slowly as compared with the rate at which bread stales, and the retrograded starch is extremely resistant to solubilization.

During the past decade our knowledge of the structure and properties of starch has greatly increased. Although earlier workers had shown that the common cereal and tuber starches could be separated by various means into two fractions which differed in their properties, methods have recently been perfected that provide a clear-cut separation into a straight chain component and a branched chain component, designated as amylose and amylopectin, respectively, by Meyer (1942). The linear component, which can be selectively precipitated from autoclaved starch solutions with butyl or amyl alcohol (Schoch, 1942), with higher fatty acids (Schoch and Williams, 1944), or with certain nitroparaffins (Whistler and Hilbert, 1945), comprises 25 to 30% of the normal starches and forms highly unstable solutions which retrograde rapidly to insoluble aggregates or irreversible gels. The retrogradation of amylose can readily occur because of the ease with which the linear molecules can orient to form

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"crystallites." On the other hand, the amylopectin, or branched chain component, forms relatively colloidally stable solutions which exhibit little tendency to retrograde. The starches prepared from the so-called waxy or glutinous varieties of cereals are unique in their properties. Pastes prepared from these "waxy" starches have high hot viscosities, low gel rigidity, and show a remarkably low tendency to retrograde (Brimhall and Hixon, 1939; Hixon and Sprague, 1942; Schopmeyer, Felton, and Ford, 1943). That waxy maize starch is composed almost entirely of the branched chain component, amylopectin, is evidenced by its low alkali number, its failure to give any precipitate with butanol (Schoch, 1942), its low iodine adsorption (Bates, French, and Rundle, 1943), and its osmotic and viscosity behavior (Foster and Hixon, 1943). The absence of any significant amount of the linear component in waxy maize starch, according to Hixon and Sprague (1942), is a possible explanation for its inability to retrograde at all rapidly since the branched molecules "would have difficulty in orienting into insoluble groups of parallel chains, which is characteristic of retrogradation."

Interpreting Katz' retrogradation theory in the light of this newer knowledge of starch chemistry, it may be postulated that bread staling is due to the amylose component of starch. If this assumption is valid, as pointed out by Hixon (1943), bread made with waxy starch should exhibit little tendency to stale. Although waxy or glutinous varieties of corn, rice, millet, and grain sorghum are known, no waxy varieties of wheat have yet been reported. However, the effect of waxy starch on bread staling can be investigated by conducting staling tests on bread made from gluten-starch mixtures. Aitken and Geddes (1938, 1939), Harris (1940), and Aitken and Anderson (1943) have shown that the baking characteristics of flours may be enhanced by the addition of crude gluten which has been dried in a stream of air at 32°C. Sandstedt, Jolitz, and Blish (1939) found that wet gluten and starch may be recombined to form a dough with baking characteristics similar to those of doughs prepared from the original flours. Harris and Sibbitt (1942) and Finney (1943) have also used the synthetic approach in studying differences in the baking value of gluten and starch prepared from different wheat varieties.

In this paper, the results of staling studies on breads made from mixtures of gluten with wheat and waxy maize starches are reported.

Materials and Methods

Preparation of Gluten and Starches. Dried gluten and wheat starch prepared from an unbleached straight grade flour milled from hard red spring wheat in connection with a previous investigation were available

for this study. The gluten was prepared by washing the starch from doughs at approximately 0°C. Part of the gluten was frozen at − 16°C, ground, and dried cryoscopically; the part used in this study was dried at room temperature (approximately 25°C) in a stream of air from an electric fan. The starch was removed from the wash water by centrifuging and divided into the upper and lower starch fractions which were frozen and dried cryoscopically. The lower starch fraction was used in preparing the synthetic flours.

Waxy maize and waxy sorghum starches prepared without the use of sulfur dioxide were supplied by the Northern Regional Laboratory, Peoria, Illinois.

Preparation of Synthetic Flours. Synthetic flours containing 12% protein (N × 5.7; 14% moisture basis) were prepared by mixing the appropriate quantities of finely pulverized dried gluten and starch. A series of flours comprising mixtures of 0, 10, 50, and 100% of waxy maize and wheat starches was prepared. In addition, mixtures of waxy sorghum and wheat starches were employed in preliminary studies.

Baking Procedure. The synthetic flours were baked by a micro-baking technique similar to that described by Geddes and Aitken (1935). The regular A. A. C. C. basic formula (*Cereal Laboratory Methods*, 4th ed., 1941) using 6% of sucrose was employed and sufficient ingredients to yield three micro loaves were mixed in a Hobart-Swanson mixer for two minutes. Upon removal from the mixer, three 50-g doughs were scaled, fermented for two hours at 30°C, molded, and proofed in low-form micro pans for 55 minutes at 30°C. The doughs were baked for 20 minutes at 230°C. The three loaves were weighed together, their combined volume determined by seed displacement, and the specific volume computed. Thirty minutes after removal from the oven, the loaves were coated with paraffin and stored in airtight tins at 26°C.

Determination of Staling. Two series of staling studies were made. In the first, the staling of the micro loaves was followed by means of the compressibility technique, as outlined by Platt and Powers (1940); in the second, the crumb swelling technique, as modified by Cathcart and Luber (1939), was used.

Compression measurements were made with the Baker compressimeter in which the usual plunger was replaced by a much smaller one, measuring 3/4 inch in diameter, thus allowing for the testing of the small slices obtained from the micro loaves. Three slices, 0.5 inch thick, were cut from a single micro loaf with the aid of a mitre box, both ends being discarded. Two separate compressions were measured on each slice, one covering the upper, the other the lower half of the

slice. During each individual determination, the depth of compression or the strain, as measured in mm, was recorded at stresses of 20 and 40 g. Tests were made on the three loaves baked from a single mix at intervals of 3, 9, and 27 hours, respectively, after baking, and were duplicated on another series of loaves baked on a different day.

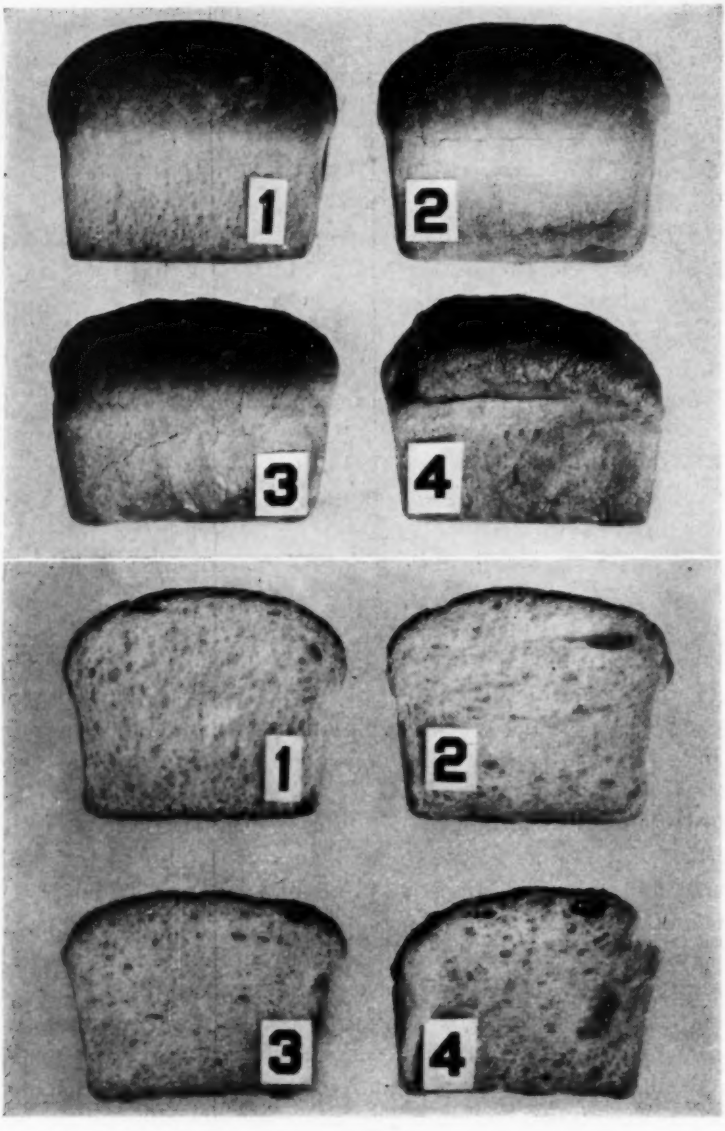

Fig. 1. External and internal views of micro loaves baked from gluten-starch mixtures. In loaf No. 1 wheat starch was used; in loaves No. 2 to 4 the starch component comprised mixtures of wheat and waxy maize starch, and contained 10, 50, and 100% waxy maize respectively.

The crumb swelling tests were made at 3 and 27 hours after baking and were duplicated on different days.

Results

Increasing the percentage of waxy maize starch in the gluten-starch mixtures increased the absorption and decreased the rate of dough development during mixing; also during fermentation the doughs tended to become dry. As can be seen from Figure 1, increasing the percentage of waxy maize starch produced a decrease in the quality of the bread. The crust became glazed and sugary in appearance and the loaf volume decreased. The crumb became less rigid, resulting in excessive shrinkage upon cooling, and was gummy in nature. These gummy characteristics were so pronounced that it was impossible to slice the bread made from gluten and all waxy maize starch, and compressibility tests could not be made.

TABLE I

EFFECT OF TIME ON COMPRESSIBILITY OF BREAD CRUMB OF LOAVES CONTAINING VARYING AMOUNTS OF WAXY MAIZE STARCH

Composition of starch mixture		Specific loaf volume	Mean compression in mm					
			Stress—20 g Elapsed time—hours			Stress—40 g Elapsed time—hours		
			3	9	27	3	9	27
Wheat %	*Waxy maize* %							
100	0	1.39	0.891	0.52	0.28	1.95	1.02	0.47
90	10	1.43	1.18	0.60	0.35	2.24	1.21	0.59
50	50	1.14	1.21	0.74	0.31	2.18	1.25	0.54

The compressibility tests recorded in Table I show that while waxy maize starch increases the compressibility of the crumb, it does not delay the rate of staling, as measured by the relative changes in compressibility with time of storage. For example, the decreases in crumb compressibility in 24 hours with a stress of 20 g were 0.61, 0.83, and 0.90 mm for 100% wheat starch, 10% waxy maize, and 50% waxy maize starch respectively. The corresponding compressibility decreases for 40 g stress were 1.48, 1.65, and 1.64 mm. Relatively large errors are involved in compressibility measurements. Noznick and Geddes (1943), working with cakes, showed that increased precision is obtained by making the tests at the higher stresses. On this basis, the results obtained at a stress of 40 g are probably the more significant, and it may be concluded that waxy maize starch has relatively little influence on the rate of hardening of bread crumb.

The low gel rigidity of waxy maize starch pastes naturally raises the question as to whether this component of the gluten-starch mixtures contributed materially to the structural rigidity of the crumb. There was an apparent slight increase in crumb compressibility of the bread containing waxy maize starch, but the increase was not progressive as the proportion of this starch was increased. As the applicability of this method of measuring staling in the instance of the gummy crumb obtained when waxy maize starch was present was open to some question, swelling tests were made as an additional measure of staling.

TABLE II

EFFECT OF TIME ON THE SWELLING CAPACITY OF BREAD CRUMB OF LOAVES CONTAINING VARYING AMOUNTS OF WAXY MAIZE

Composition of starch mixture		Specific loaf volume	Volume of centrifugate [1]		Difference in volume
			Elapsed time—hours		
			3	27	
Wheat %	*Waxy maize* %	*cc*	*ml*	*ml*	*ml*
100	0	1.13	4.6	4.1	0.5
90	10	1.20	4.4	4.1	0.3
50	50	0.95	6.0	3.8	2.2
0	100	0.92	7.2	5.4	1.8

[1] Volume for 2 g of crumb (original moisture basis).

The results, recorded in Table II, show that waxy maize starch increases the swelling capacity of the crumb when compared at the same time interval after baking. The presence of this starch resulted in a greater decrease in swelling capacity over the 24-hour time interval than occurred with wheat starch. It is noteworthy, however, that the actual swelling capacity of the cumb in which the starch component consisted entirely of waxy maize starch was somewhat higher after 27 hours' storage than the crumb for all wheat starch after 3 hours' staling. The washings obtained from the crumb became progressively more slimy as the percentage of waxy maize starch was increased.

Discussion

Though limited in scope, these studies show quite definitely that waxy maize starch has a very detrimental effect on dough and bread quality and is of no value in decreasing the rate of staling. In fact, as measured by the change in crumb swelling capacity, the staling rate is increased. Though less complete, additional studies employing waxy sorghum starch gave similar results to those obtained with waxy maize starch.

As waxy starches are essentially free of amylose, the results indicate that the staling of bread at least cannot be solely attributed to the marked retrogradation tendencies of this component of wheat starch. The ease with which stale bread can be freshened, such as by heating at a temperature of approximately 60°C for a few minutes, is not in line with the postulate that the amylose fraction is the primary factor, since retrograded amylose must be subjected to prolonged autoclaving to bring it into solution.

Since the swelling capacity of the crumb in which the starch component consisted only of waxy maize starch markedly decreased with age, it would appear that changes in the amylopectin component of wheat starch may well be a primary factor in the bread staling process if it is assumed that the properties of the amylopectin from these two sources are similar. Such a view is difficult to reconcile with published researches showing that amylopectin solutions exhibit little tendency to retrograde. Yet, it is quite conceivable that retrogradation of the branched chain component of wheat starch may occur under the conditions existing in bread. Retrogradation studies have usually been limited to solutions containing less than 10% of starch which is many times less than that in bread. Schoch (1941) has pointed out that the rate of retrogradation of starch solutions increases with concentration. In spite of the difficulty of highly branched molecules to orient into a crystalline-like structure, aggregation of amylopectin molecules may conceivably occur in bread where the starch is considered to have undergone only a restricted type of gelatinization and where the high gel concentration increases the probability of alignment of the branches.

Summary

Bread was baked from gluten-starch mixtures, the starch component of which consisted of mixtures of wheat starch with various quantities of waxy maize starch. The rate of staling was followed by compression and swelling tests on the crumb.

Flour absorption and dough development time increased, and specific volume and crumb rigidity decreased, with an increase in the percentage of waxy maize starch present. Complete substitution of waxy maize starch for wheat starch gave loaves which were characterized externally by a shiny, sugary crust and internally by a gummy, compressible crumb and excessive shrinkage after baking.

Crumb compressibility and crumb swelling capacity, measured three hours after baking, were increased when the starch of waxy maize was substituted for that of wheat. Upon storage, crumb compressibility of loaves containing waxy maize decreased at about the same rate as that of loaves made from mixtures of gluten and wheat

starch. Swelling capacity of the crumb decreased more rapidly with time when waxy maize starch was present.

These studies suggest that bread staling is associated with changes in the branched chain component (amylopectin) of wheat starch.

Acknowledgment

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EFFECT OF SOY FLOUR AND NONFAT DRY MILK SOLIDS IN WHITE BREAD ON THE NUTRITIONAL QUALITY OF THE PROTEIN AS MEASURED BY THREE BIOLOGICAL METHODS

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The nutritional quality of white bread [1] has received considerable study, especially during the recent war years, and the resulting "enriched bread" program has undoubtedly improved the American diet

[1] The terms white bread and whole wheat bread, as used throughout this paper, refer to the products defined in the proposed order of the Federal Security Agency (August 3, 1943). In referring to white bread or whole wheat bread made without milk or other optional proteinaceous materials, the terms white water bread and whole wheat water bread are used.

with respect to the added vitamins and iron. The protein of white bread is another subject receiving considerable attention at present.

Numerous experiments, using both human and animal subjects, have demonstrated that the protein of whole wheat flour and bread, in spite of lower digestibility, has superior nutritional quality to that of white flour and white water bread. Publications of the following are cited as examples: Murlin, Marshall, and Kochakian (1941), Rostorfer, Kochakian, and Murlin (1943), Chick (1942), reviews by Boas Fixsen (1935), and Copping (1939). French and Mattill (1935) found no difference in biological value between white and whole wheat breads, but their work is not strictly comparable with the above, since their "whole wheat" bread was 50% white flour and both breads contained an unspecified amount of nonfat dry milk solids.[2]

The chief deficiency of wheat protein is the amino acid, lysine. This was shown as early as 1914 by Osborne and Mendel (1914) and was confirmed by Mitchell and Smuts (1932), using paired feeding. Light and Frey (1943), using ad libitum growth tests with rats, demonstrated that lysine is the primary amino acid deficiency and valine the secondary.

Nonfat milk solids, which supply lysine and valine, have been used rather extensively by the baking industry in white bread in percentages up to 6% of the flour. Fairbanks (1939), using paired feeding, Light and Frey (1943), using ad libitum feeding, and Mitchell, Hamilton, and Shields (1943), using both feeding techniques, have shown that 6% nonfat milk solids effect improvement in the growth-promoting properties of white water bread. This same improvement was effected in white flour in an ad libitum feeding trial by Hove, Carpenter, and Harrel (1945). Mitchell *et al.* (1943) and Light and Frey (1943) found 6% white milk bread [3] equal to whole wheat water bread in their trials.

The protein of soy flour, due to its lysine and valine content, may also be used to increase the nutritional quality of white flour protein. The lysine content of soy protein is 5.4% and that of milk protein is 7.5%; the valine content of both proteins is identical, 4.5%. Defatted soy flour, however, contains about one and one-half times as much protein as nonfat milk solids. Therefore, defatted soy flour actually contributes an equal amount of lysine and more valine than nonfat milk solids when similar percentages of either are incorporated into bread. This is illustrated below:

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[2] Nonfat dry milk solids will be referred to hereinafter as nonfat milk solids.

[3] The term 6% white milk bread refers to bread made with 100 parts of white flour plus 6 parts of nonfat milk solids. Likewise the term 5% white soy bread means bread made with 100 parts of white flour plus 5 parts of soy flour. Three percent white milk bread and 3% white soy bread are similarly defined.

	Protein %	Lysine %	Valine %
Soy flour, defatted	50.0	2.7	2.3
Nonfat milk solids	35.0	2.6	1.6
White water bread	13.0	0.19	0.33
3% white soy bread	14.5	0.27	0.38
3% white milk bread	13.9	0.27	0.36
5% white soy bread	15.3	0.33	0.41
6% white milk bread	14.8	0.33	0.40

The amino acid figures for the proteins of soy flour, nonfat milk solids, white flour,[4] and bakers' yeast [5] were taken from Block and Bolling (1945) and were used to calculate the amino acid content of the breads.

Earlier nutritional studies on the use of soy flour in bread, reviewed by Horvath (1938), demonstrated the definite supplemental effect of 10% to 30% soy flour. Recently work has been done on the contribution of low percentages of soy flour to the nutritional quality of bread protein.

Jones and Divine (1944), using an ad libitum growth test, compared the supplementary value of several proteins to white flour. They reported their data in the form of both total gain in body weight and protein efficiency.[6] They placed emphasis on the gains in body weight, and so concluded that 5% expeller soy flour was inferior in supplementary value to 5% nonfat milk solids when combined with 95% white flour. However, their results for protein efficiency showed that the 5:95 soy flour and the 5:95 nonfat milk solids mixtures with white flour were equal. It seems more valid to emphasize their protein efficiency data, since the food intake was not equalized. The results of Harris, Clark, and Lockhart (1944), who used an ad libitum growth test, showed that a 3% white soy bread made with full-fat soy flour was equal to a 4% white milk bread in the nutritional quality of protein, both breads being superior to white water bread. Their 5% white soy bread was equal to their 6% white milk bread in the same test. In addition, they found a bread containing both 3% full-fat soy flour and 3% nonfat milk solids superior to either 5% white soy bread or 6% white milk bread. Volz, Forbes, Nelson, and Loosli (1945), using both a paired feeding growth test and nitrogen balance technique, showed that 5% full-fat soy flour improved the protein efficiency and biological value of the protein of 3% white milk bread.

Biological methods for measuring differences in nutritional quality between proteins from various sources are of two types: nitrogen balance studies, in which dietary nitrogen retention is the basis for

[4] 1.9% lysine and 3.4% valine in white flour protein.
[5] 6.4% lysine and 4.4% valine in bakers' yeast protein.
[6] The term protein efficiency is used in this paper to designate grams gain in weight per gram of protein eaten, sometimes referred to as nutritive value of protein.

evaluating proteins, and animal growth studies, in which body protein gain and protein efficiency may be taken as the measure. Two methods of feeding have been used in the latter type of test—ad libitum and equalized. All methods have been subject to criticism and their limitations and advantages have been discussed by Barnes, Maack, Knights, and Burr (1945), and Mitchell (1944), authorities representing different points of view.

The purpose of the experiments reported herein was to determine by the various biological methods the nutritional quality of the protein in white water bread, white bread containing low levels of soy flour, white bread containing similar levels of nonfat milk solids, and whole wheat water bread. This procedure was adopted in order to establish reliability of the results, in view of the differences of opinion on methods of evaluating the nutritional quality of food proteins.

Materials and Methods

Three experiments were included in this investigation. Experiment I was a growth assay using ad libitum feeding. Experiment II was a growth assay in which food intake was equalized. Body protein storage measurements were included as part of the latter assay. Experiment III was a nitrogen balance study.

Six types of experimental breads were used: white water bread, 3% white soy bread, 5% white soy bread, 3% white milk bread, 6% white milk bread, and whole wheat water bread. All breads except the whole wheat water bread were made from enriched flour.[7] The experimental breads were incorporated into the diets at levels to provide 10% protein (N × 6.25).[8] These diets, formulated for nutritional completeness with respect to all known nonprotein nutrients, derived their protein solely from the breads.

Preparation of the Breads. The breads used in these studies were prepared in laboratory equipment using a straight-dough mix. The formula consisted of enriched white flour (80% patent) 100 parts, compressed yeast 2 parts, sugar 3 parts, salt 2 parts, shortening 2 parts, Arkady [9] 0.45 part, and water as determined by absorption.[10] The stipulated number of parts of soy flour and nonfat milk solids was added to 100 parts of enriched white flour.

The enriched white flour and the whole wheat flour were made from the same blend of hard spring wheat. The enriched white flour

[7] The enrichment complied with minimum requirements as published by the Federal Security Agency (July 3, 1943), which are: thiamine 2.0 mg, riboflavin 1.2 mg, niacin 16 mg, iron 13 mg per pound of flour.

[8] Since there was no one factor appropriate for all the breads tested, the 6.25 factor was used in all calculations involving conversion of Kjeldahl N to protein in this paper, as in the publication of Hove *et al.* (1945).

[9] A mixture of NH_4Cl, $CaSO_4$, $KBrO_3$, and a starch filler used as a dough improver.

[10] Enriched white flour had an absorption of 66%. The other flour mixes had higher absorptions.

was bleached with Novadel (active principle, benzoyl peroxide) and Agene (nitrogen trichloride) according to commercial practice. The soy flour was of the defatted [11] type and the nonfat milk solids were a spray-dried product; both were made expressly for bakery use. The analysis of these bread ingredients is given in Table I.

TABLE I

ANALYSIS OF BREAD INGREDIENTS

Ingredient	Type	Moisture	Protein	Fat [1]	Ash
		%	%	%	%
Enriched white flour	Hard spring	13.1	12.8	0.9	0.4
Nonfat milk solids	Spray dried	5.3	37.1	0.7 [2]	—
Soy flour	Defatted	7.3	50.6	1.2	5.8
Whole wheat flour	Hard spring	12.1	15.3	2.1	1.8

[1] Ether extract following acid hydrolysis. [2] Rohrig tube method of Roese-Gottlieb.

The doughs were mixed in a Hobart Mixer for 1 minute at low speed and for 4 minutes at medium speed. When delivered from the mixer, they had an average temperature of 80°–82°F. The fermentation schedule was first punch $1\frac{1}{2}$ hours, to bench $\frac{3}{4}$ hour, to molder $\frac{1}{4}$ hour; total fermentation time $2\frac{1}{2}$ hours at 80°F. The fermented doughs were panned, proofed 60 minutes at 90°F, and baked 30 minutes at 425°F in a rotary-hearth type oven. The freshly baked breads were sliced, air-dried, and ground to a powder of approximately 80 mesh. Table II contains the analysis of the dried breads as they were used in the diets.

TABLE II

ANALYSIS OF DRIED BREADS

Description of breads			Moisture	Protein	Fat [2]	Ash
Bread flour	Protein supplement	Parts [1]	%	%	%	%
Enriched white	None		9.6	13.0	3.0	2.5
Enriched white	Nonfat milk solids	3	8.4	13.9	3.3	2.6
Enriched white	Nonfat milk solids	6	7.5	14.8	3.4	2.6
Enriched white	Soy flour	3	7.0	14.5	3.5	2.6
Enriched white	Soy flour	5	6.9	15.3	3.0	2.6
Whole wheat	None		7.2	14.2	3.6	2.9

[1] Parts added to 100 parts of white flour. [2] Ether extract following acid hydrolysis.

Preparation of the Diets. The diets were formulated according to the following basic pattern: dried bread to supply 10% protein,

[11] Hexane extracted.

minerals [12] 4.0%, cod liver oil 2.0%, celluration [13] to equalize crude fiber at approximately 2%, soybean oil to equalize fat at 6%, and corn starch to make up the balance of the diets. In Experiment I, 4.0% of B-complex vitamin solution [14] was incorporated into the diets. In Experiments II and III each rat received orally 0.4 ml of the B-complex vitamin solution every day. Mixtures of dry ingredients, equivalent to 6 kilograms of complete diet, were prepared for each lot. Kilogram quantities were mixed with the liquid ingredients as needed. Protein analyses of the complete diets are given in column 2 of Table III. The diets were compounded according to the same formula throughout the investigation..

Feeding and Experimental Procedures. The rats used for all tests were weanling male albinos of the Sprague-Dawley strain, 24 to 25 days old and weighing 40 to 55 g. Within any one of the three assays to be described the animals did not vary more than 10 g in weight. They were individually caged and given access to water. Cages were located in a well-lighted, air-conditioned animal room. Temperature was maintained at 75° ± 2°F at a relative humidity of 50% to 70%. The procedure for each of the experiments is described below:

I. Growth Assay—Ad Libitum Feeding. This experiment was a 56-day growth assay using a modification of the method of Osborne, Mendel, and Ferry (1919). Thirty-six rats were distributed at random into six lots of six animals each. Each animal was weighed during the same hour on three consecutive days at the start to obtain average initial weight, and at 7-day intervals for 8 weeks thereafter. Final weights were determined in the same manner as were the initial weights.

The diets were offered ad libitum in cups designed to minimize spillage. The food was weighed fresh each day, and the rejected food from the previous feeding was discarded. Individual food consumption records were averaged at 7-day intervals for each lot. The total weight of protein consumed per rat during the 56-day assay period was determined by multiplying the total feed consumed by the actual percentage of protein in the diet.

II. Growth Assay—Equalized Intake. Technique for this assay was the same as described above, except that the total food intake of all animals in this assay was equalized with that of the rat which consumed the least, provided that this rat was not obviously abnormal. This assay was conducted for 63 days. Protein efficiency data were obtained at the end of 56 days; body protein storage was determined at the end of 63 days.

Body Protein Storage. To obtain analytical data on the body protein gains of the rats, the carcass of each animal was opened, the digestive tract removed, and washed free of its contents. The unground carcasses and digestive tracts of the six animals in each lot were then digested in about 3,000 ml of 20% H_2SO_4 until a uniform solution resulted. Digests were diluted to volume and aliquots analyzed for total nitrogen content as described in A.O.A.C. *Official Methods of Analysis* (1940). Eleven weanling males were analyzed by the above procedure to obtain the initial average protein content of the rats; body protein gains were calculated on the basis of these determinations.

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[12] Osborne-Mendel-Wesson Salt Mixture (Wesson, 1932) modified to contain 0.13 g of $ZnSO_4 \cdot 7\ H_2O$ per kilogram.

[13] Processed rice hulls, analyzing approximately 65–70% crude fiber and 0.0% protein and purchased from Fisher Scientific Company, Chicago, Illinois.

[14] Thiamine, riboflavin, and pyridoxine 300 μg each, niacin 2.5 mg, calcium pantothenate 1.1 mg, para-aminobenzoic acid 7.5 mg, and choline hydrochloride 16.6 mg dissolved in 4 ml of water and used on the basis that 4 ml is equal to 4 g.

TABLE III

Summary of Experimental Data and Results

Diet No.	Protein (N×6.25) in diet	Source of dietary protein			Growth studies [5]							Nitrogen balance study			
					Ad libitum intake			Equalized intake				True digestibility		Biological value	
		Bread flour	Protein supplement		Weight gain	Protein eaten	Protein [6] efficiency	Weight gain	Protein eaten	Protein [6] efficiency	Body protein gain [7]	Part A	Part B	Part A	Part B
	%			*Parts* [2]	g	g		g	g		g	%	%	%	%
392	10.8	White [1]	None		36.0	41.6	0.87	19.1	29.0	0.66	4.2	93.8	92.9	39.2	38.9
393	10.4	White [1]	N.M.S. [3]	3	42.6	42.3	1.01	23.7	28.6	0.83	5.0		94.6		45.6
394	10.4	White [1]	N.M.S. [3]	6	47.7	44.7	1.07	25.9	28.6	0.91	6.0		94.2		49.0
401	10.4	White [1]	Soy flour [4]	3	44.6	44.5	1.00	24.5	28.6	0.85	5.2		93.6		46.5
402	10.2	White [1]	Soy flour [4]	5	58.3	47.1	1.24	26.3	28.6	0.93	5.9	91.7		50.6	
411	10.4	Whole wheat	None		55.5	47.2	1.18	21.5	28.5	0.75	4.2		92.9		45.9
Pooled standard error of means							0.032 N=6			0.042 N=6	0.352 N=6			1.25 N=8	1.43 N=10
Least significant difference between any two means:			Odds 19 : 1				0.12			0.15	1.28			4.19	4.57
			Odds 99 : 1				0.19			0.23	2.01			6.19	6.57

[1] Enriched.
[2] Parts added to 100 parts of white flour.
[3] Nonfat milk solids.
[4] Defatted type.
[5] Basis 56 days.
[6] Grams gain in weight per gram of protein eaten, sometimes referred to as nutritive value of protein.
[7] Basis 63 days.

III. Nitrogen Balance Test. The biological value and digestibility for each protein were determined using the technique of Mitchell and Carman (1926), with modifications described by Mitchell (1943). The nitrogen balance experiment was carried out in two parts. Part A, using eight rats, was a comparison between 5% white soy bread and white water bread. This test involved an initial standardization period during which a nitrogen-free ration (0.062% N) was fed to all rats for a 4-day preliminary period, and for a 3-day test period in which urine and feces were collected. The animals were then divided into two groups of four rats each, and each group was placed on one of the test rations for a 4-day preliminary and a 7-day collection period. Following this, the diets were reversed for a similar period. Food intake was equalized throughout the test in the same manner as for the growth test with equalized feeding.

Part B was a comparison of white water bread, 3% white soy bread, 3% white milk bread, 6% white milk bread, and whole wheat water bread. Biological values for the protein in these breads were determined using 10 rats for each bread. This test was conducted in the same manner as Part A, except that a final nitrogen-free period, similar to the initial one, was run after the two periods in which the test diets were fed. An average of initial and final endogenous nitrogen was used in calculating biological values. This variation was incorporated to reduce the relatively high endogenous nitrogen, and subsequently higher biological values obtained when using only an initial nitrogen-free period, as reported by Olson and Palmer (1940). White water bread was included in both Parts A and B so that a comparison of values by both procedures could be made.

Results and Discussion

The results of all the experiments are shown in Table III.[15] Statistical analyses of the data were made by the method of Snedecor (1940), using covariance analysis for the ad libitum growth study and analysis of variance for both evaluations of the equalized feeding assay. Brandt's method of testing significance in reversal experiments, described by Snedecor, was used for the nitrogen balance test.

All the methods used showed that the proteins of the two white soy breads and the two white milk breads were superior in nutritional quality to the protein of white water bread. As measured by the biological values and the protein efficiencies from both growth assays, the differences between white soy or white milk breads and white water bread were in all cases statistically significant. The body protein gains, food intake being equalized, were significantly higher for 5% white soy and 6% white milk breads than for white water bread; the body protein gains for 3% white soy and 3% white milk breads were also higher than for white water bread, although these latter dife ferences were not statistically significant.

The 3% white soy bread was equal in the nutritional quality of the protein to 3% white milk bread, both breads being superior to whit-water bread, as determined by all three experimental methods. Our results for these breads are given below with the results of other investigators:

[15] Complete data for the nitrogen balance test may be obtained from the authors on request.

	Biological value			Protein efficiency				Body protein gains
				Ad libitum intake		Equalized intake		
	This paper	Henry *et al.* (1941)	Volz *et al.* (1945)	This paper	Harris *et al.*[1] (1944)	This paper	Volz *et al.* (1945)	This paper
	%	%	%					g
Bread								
White water bread	39.2 (A) 38.9 (B)	44.7		0.87	0.51	0.66		4.2
2% White milk bread	—	47.6		—	—	—		—
3% White milk bread	45.6		43.3	1.01	—	0.83	0.97	5.0
3% White soy bread	46.5			1.00	0.69	0.85		5.2
4% White milk bread	—			—	0.67	—		—
				Jones & Divine (1944)	Hove *et al.* (1945)			
Flour								
White flour				0.75	0.84		0.80	
White flour, 97 parts +N.M.S.,[2] 3 parts					1.00			

[1] The values reported by Harris *et al.* (1944) were converted to protein efficiency values by dividing by the factor 6.25 for convenience in comparing data.
[2] Nonfat milk solids.

The protein of 5% white soy bread was found equal to the protein of 6% white milk bread in nutritional quality when comparing biological values, protein efficiencies, and body protein gains, using equalized feeding. The protein efficiencies for these same breads in the ad libitum assay showed 5% white soy bread to be significantly better than 6% white milk bread, although neither bread differed significantly from whole wheat water bread. A comparison of our results with those reported in the literature is tabulated on page 314.

Since our protein efficiency results for 5% white soy bread and 6% white milk bread in the ad libitum feeding test do not agree with those reported by Jones and Divine (1944) with flours or Harris *et al.* (1944) with breads, the possibility that heat of baking may have had some effect on the performance of soy and milk proteins is receiving consideration.

In any of the experiments included in our study, the nutritional quality of the protein in either 5% white soy or 6% white milk bread did not differ statistically from that in either 3% white soy or 3% white milk bread, with one exception. This was in the ad libitum growth assay in which the protein efficiency of 5% white soy bread was significantly higher than that of the other white breads. However, biological values and protein efficiencies from both growth assays showed that there was a significant (odds 19:1) difference between 3%

	Biological value			Protein efficiency				Body protein gains
				Ad libitum intake		Equalized intake		
	This paper	Henry *et al.* (1941)	Volz *et al.* (1945)	This paper	Harris *et al.* (1944)	This paper	Volz *et al.* (1945)	This paper
	%	%	%					g
Bread								
5% White soy bread	50.6			1.25	0.75	0.93	—	5.9
6% White milk bread	49.0	49.7		1.07	0.76	0.91	—	6.0
White (3%) milk (5%) soy bread	—		47.7	—		—	1.17	—
Whole wheat water bread	45.9			1.18		0.75	—	4.2
				Jones & Divine (1944)	Hove *et al.* (1945)	Barnes *et al.* (1945)		
Flour								
White flour, 95 parts +Soy flour, 5 parts				1.38	—			
White flour, 95 parts +N.M.S.,[1] 5 parts				1.44	—		1.13	
White flour, 94 parts +N.M.S.,[1] 6 parts				—	1.19			
Whole wheat flour				1.15	1.40	0.42		

[1] Nonfat milk solids.

white soy or 3% white milk bread and white water bread proteins, while there was a highly significant (odds 99:1) difference between 5% white soy or 6% white milk bread and white water bread proteins. The body protein storage data showed this same trend. Therefore, our results by all biological methods showed that the nutritional quality of the protein in white bread increased as the percentage of soy flour or nonfat milk solids added to the bread was increased from 3% to 5% or 6%.

The biological values of the proteins showed whole wheat water bread to be superior to white water bread, equal to 3% white soy or 3% white milk bread, but slightly inferior to 5% white soy or 6% white milk bread. In our ad libitum growth assay, the protein efficiency of whole wheat water bread was significantly higher than the protein efficiencies of white water bread, 3% white soy, or 3% white milk bread, but did not differ significantly from those of 5% white soy or 6% white milk bread. However, in the growth assay employing equalized food intake, the protein efficiencies and body protein gains for whole wheat water bread did not differ significantly from those for white water bread. Under the same conditions, the protein efficiencies and body protein gains of whole wheat water bread were slightly lower than those of 3% white milk or 3% white soy bread,

and significantly lower than those of 6% white milk or 5% white soy bread.

These results are in contrast to those of Mitchell *et al.* (1943) who, in their paired feeding assay as well as in their ad libitum assay, found equal growth-promoting value for whole wheat water bread and 6% white milk bread, white water bread being inferior. They were, however, feeding the entire breads plus cod liver oil and hence a higher, as well as a more variable, protein level than was used in our study. The protein analyses for their white water bread, 6% white milk bread, and whole wheat water bread were 14.06%, 14.75%, and 15.75%, respectively.

Our results are in agreement with those of Barnes *et al.* (1945) who reported a lower protein efficiency for whole wheat flour when food intake was restricted than when their animals were fed ad libitum. In their trial, the food intake of each rat was restricted by paired feeding for 54 days and then a period of ad libitum feeding was begun. The results we obtained for whole wheat water bread in our ad libitum and equalized feeding trials confirm their results as shown below:

	Protein in diet	Type of feeding	Daily food intake	Protein efficiency
	%		g	
This paper	10.4	Restricted	5.1	0.75
		Ad libitum	7.4	1.18
Barnes *et al.* (1945)	11.2	Restricted	3.44	0.42
		Ad libitum	7.46	1.03

The nutritional quality of the protein of whole wheat water bread apparently varies with the type of feeding and the level of protein in the diet. In our assay the use of different biological methods resulted in a change in relative rank of the protein in whole wheat water bread when compared with the proteins in the other breads as to nutritional quality. Whole wheat protein probably does not furnish sufficient amino acids for growth when the food intake is considerably restricted on a low (10%) protein diet.

Summary

Three biological methods were used to evaluate the nutritional quality of the protein present in white water bread, 3% and 5% white soy breads, 3% and 6% white milk breads, and whole wheat water bread. The three biological methods used were as follows: nitrogen balance; two growth assays, one using ad libitum and the other equalized food intake; and body protein storage.

The results for the nutritional quality of the bread protein obtained by the three methods showed that 3% white soy bread and 3% white milk bread were equal, and that both of these breads were significantly better than white water bread. The 5% white soy bread was at least equal to 6% white milk bread, and both of these were slightly superior to the 3% white soy or 3% white milk breads, and significantly superior to white water bread. The 5% white soy bread was equal to or better than whole wheat water bread, as was 6% white milk bread, except in the ad libitum growth assay where the difference was not significant.

The three methods showed good agreement with respect to the relative rank of the five white bread proteins, while the relative position of whole wheat water bread protein was apparently related to the level of food intake.

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THE VITAMIN B-COMPLEX CONTENT OF THE COMMERCIAL MILLING PRODUCTS OF EGYPTIAN RICE

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Kik and Van Landingham (1943, 1944), Williams, Knox, and Fieger (1943), and Kik and Williams (1945) have published extensive figures for the vitamin B-complex content of many varieties of rice grown and milled in the principal rice-producing localities of the United States. No similar data appear to be available for Egyptian rice, and the present investigation was undertaken at the suggestion of the Ministry of Food in September, 1944. Interest lay primarily in the distribution of the vitamins of the B_2 complex, but a few figures for thiamine (Vitamin B_1) were also obtained.

Materials and Methods

The paddy used was Egyptian-grown from a hybridized Japanese-type seed. With the exception of the tail-ends, all the samples were drawn from a single bulk mill lot of 12.5 tons of paddy.

Thiamine was determined by the method of Harris and Wang (1941).

Pyridoxine was determined by the method of Stokes *et al.* (1943), using the "pyridoxinless" mutant (No. 299) of *Neurospora sitophila* as test organism.

Riboflavin was determined by the method of Snell and Strong (1939), using *Lactobacillus helveticus*, as modified by Barton-Wright and Booth (1943). Extracts were prepared by autoclaving 1 to 5 g of the finely ground material with 50 ml 0.1 *N* hydrochloric acid for 15 minutes at 15 pounds per square inch, followed by precipitation and filtration at pH 4.5 as recommended by Strong and Carpenter (1942). This latter modification is essential for the assay of cereals with the media at present in use, as it minimizes the "drift" so frequently observed in the assay of substances of this type. Even so, some non-specific growth-stimulation is frequently obtained at the lower riboflavin levels, and it is considered that the technique is still capable of improvement. Precautions were taken to ensure uniformity of incubation temperature, as stressed by Price and Graves (1944), and the tests were incubated for 72 hours in a mechanically stirred water bath at 40 ± 0.05°C.

Nicotinic Acid was determined by a microbiological method using *Lactobacillus arabinosus*. The medium was that described by Snell and Wright (1941), although in subsequent work the modifications proposed by Krehl, Strong, and Elvehjem (1943) have been adopted. Extracts of samples for assay were prepared by autoclaving 1 to 5 g of the finely ground material with 50 ml *N* hydrochloric acid for 15 minutes at 15 pounds per square inch. After cooling, the pH was adjusted to 6.8, the extracts suitably diluted for assay and filtered. In our experience this has proved one of the most satisfactory of the microbiological methods. Agreement between the titrations on duplicate tubes is consistently good, and excellent reproducibility is obtained when the sample is assayed on different occasions.

Results and Discussion

The values obtained are shown in Table I. Since the proportions by weight of the by-products and of the finished rice are known, it is of interest to investigate the distribution, during the milling process, of the vitamins originally present in the paddy. The distribution of nicotinic acid, pyridoxine, and riboflavin is shown in Table II.

TABLE I

VITAMIN B-COMPLEX CONTENT OF RICE MILLING FRACTIONS

Sample	Vitamin content			
	Nicotinic acid	Pyridoxine	Riboflavin	Thiamine
	μg/g	μg/g	μg/g	μg/g
Paddy	50.4	8.1	0.66	—
Loonzain (brown rice)	50.6	—	—	—
Hulls (husks)	9.2	3.6	0.49	—
1st Coning Rice	33.4	—	—	—
2nd Coning Rice	20.9	—	—	—
Meal from 1st Coning	506.0	63.0	2.2	34.8
Meal from 2nd Coning	365.0	52.0	2.6	36.9
Fannings [1]	98.8	14.6	1.2	6.9
Dusty chaff [1]	128.0	14.5	1.2	4.2
Brokens [1]	42.6	5.7	0.76	—
Tail ends	57.5	5.5	0.55	—
Finished rice	17.6	3.4	0.35	—

[1] The terms "fannings" and "dusty chaff" are colloquialisms used by the miller for by-products composed mainly of meal and fine husks. "Brokens" includes both the "screenings" and "brewer's rice" of American milling practice.

The results are in general agreement with those for American-grown rice published by Kik and Van Landingham (1943 and 1944) and Williams, Knox, and Fieger (1943). Our value for the nicotinic acid content of hulls (9.2 μg/g), however, is lower than that found by

TABLE II

DISTRIBUTION OF B-COMPLEX VITAMINS IN THE RICE MILLING PROCESS

Sample	% by weight of paddy	Contribution made by each fraction per 100 g of paddy			% of original vitamins retained in each fraction		
		Nicotinic acid	Pyridoxine	Riboflavin	Nicotinic acid	Pyridoxine	Riboflavin
Paddy	% 100	μg 5040	μg 810	μg 66	% 100	% 100	% 100
Hulls (husks)	16.01	148	58	7.8	2.9	7.2	11.8
Meal from 1st Coning	3.3	1670	208	7.3	33.1	25.7	11.1
Meal from 2nd Coning	3.12	1139	162	8.1	22.6	20.0	12.3
Fannings	1.36	134	20	1.6	2.7	2.5	2.4
Dusty chaff	1.36	174	20	1.6	3.5	2.5	2.4
Brokens	3.32	142	19	2.5	2.8	2.3	3.8
Tail ends [1]	7.72	443	42	4.2	8.6	5.2	6.4
Finished rice	60.88	1072	207	21.3	21.5	25.6	32.3
Weight loss on milling	2.93						
Totals	100	4922 (97.7%)	736 (91.0%)	54.4 (82.5%)	97.7	91.0	82.5

[1] This sample derived from a different mill lot.

Kik and Van Landingham (1944), who reported 14.0–25.1 μg/g. Our nicotinic acid figures for the first and second coning meals (506 and 365 μg/g) are higher than these authors' findings (303.2–358.2 and 259.5–316.0 μg/g); they approximate more closely those of Williams, Knox, and Fieger (1943) for rice bran (334.8–487.4). We find higher thiamine and pyridoxine values (34.8 and 36.9 μg thiamine per gram and 63 and 52 μg pyridoxine per gram) than were reported by Williams *et al.* (1943) for rice bran (26.6–30.1 μg thiamine per gram and 23.8–38.6 μg pyridoxine per gram). Kik and Van Landingham (1943) report only 0.25–0.41 and 0.25–0.49 μg riboflavin per gram in screenings and brewer's rice respectively, while 0.76 μg/g was found for broken rice in the present work.

The riboflavin is more evenly distributed throughout the grain than is the nicotinic acid; pyridoxine is intermediate in this respect. A similar observation regarding the distribution of nicotinic acid and riboflavin in wheat has been made by Jackson, Doherty, and Malone (1943).

The apparent difference between the total riboflavin content of the various fractions in Table II and the riboflavin content of the whole paddy is within the limits of experimental error for samples of such relatively low potency. The "weight loss" (2.93%) is abnormally high, owing to the fact that the samples were drawn on the first day of milling.

Summary

The nicotinic acid, pyridoxine, and riboflavin contents of Egyptian paddy and milling fractions are in substantial agreement with figures published for rice grown and milled in the United States.

Of the nicotinic acid, pyridoxine, and riboflavin present in the original paddy, 21.5, 25.6, and 32.3% of these factors respectively were retained in the finished rice, and 55.7, 45.7, and 23.4% were removed as rice meal.

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THE QUANTITATIVE DETERMINATION OF MOLDS IN FLOUR [1,2]

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Methods to determine the number of microorganisms in flour have been described by Kent-Jones and Amos (1940), and by Barton-Wright (1938), and have been included in *Cereal Laboratory Methods* (4th ed., 1941). These methods were designed principally to assay the number of bacteria, and while they presumably would apply equally well to the molds, there has been almost no attempt to determine whether they actually would permit an accurate mold census to be made of a given flour sample.

The present study was undertaken to determine some of the chief factors that influence the number of molds cultured from a given flour sample. Preliminary work with samples taken from different streams in different mills, and with sterile flour to which known molds were added, indicated that the most important variables involved were: the composition of the medium on or in which the flour was cultured; the technic of making the dilutions; and the method of counting the number of colonies in the culture dishes. Therefore most of our efforts were concentrated on these three variables.

Experimental

Culture Media. About 10 culture media were tested, the following ones fairly extensively: Smith-Humfeld agar, designed by Smith and Humfeld (1930) to facilitate the isolation of a large variety of organisms from soil; a penicillin assay medium essentially the same as the Medium No. 1 described by Schmidt and Moyer (1944) but containing also 1.5 g of beef extract per liter; potato dextrose agar; potato dextrose agar acidified by the addition of 0.5 ml of 50% lactic acid per 100 ml of agar just before pouring into plates; malt agar containing 2% malt extract and 2% agar per liter; and a malt-salt agar made as follows:

Malt extract (any of the several commercial brands tested were suitable)	20.0 g
Sodium chloride	75.0 g
Agar, crude shredded	20.0 g
Distilled water to make	1.0 liter

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[2] This investigation was aided by a grant from Wallace and Tiernan Company, Inc., Newark, New Jersey.

The details of preparation of this medium are given later in the paper. It is somewhat acid (about pH 5), and the salt concentration is high enough to inhibit completely the growth of any bacteria so far encountered in flour. Common species of *Rhizopus*, *Mucor*, *Alternaria*, *Penicillium*, and *Aspergillus* grow relatively slowly upon it. Such common and abundant inhabitants of flour as *Aspergillus candidus* and *A. glaucus* grow more rapidly and appear in greater numbers on the malt-salt medium than upon any of the other media tested.

Two flour samples, one containing a relatively small number of a few species of molds, the other containing a relatively large number of a greater variety of molds, were assayed on these five media, using the method described in detail later in the paper. The results are given in Table I.

TABLE I

INFLUENCE OF THE CULTURE MEDIUM ON THE NUMBER OF MOLDS CULTURED FROM TWO SAMPLES OF FLOUR [1]

Medium	Mold colonies per gram of flour	
	Sample 1	Sample 2
Penicillin assay medium	160	—[2]
Potato-dextrose	320	960
Smith-Humfeld	550	3800
Acid potato-dextrose	1180	3800
Malt-salt	1800	5900

[1] Each figure is an average of five plates.
[2] Almost no molds appeared on this agar; all five plates were overgrown with bacteria.

Similar results were obtained in other tests. In most cases a given sample of flour yielded 50% more mold colonies on malt-salt agar than on Smith-Humfeld or acid potato-dextrose agar, and often yielded 1000% more than upon the penicillin assay medium or ordinary potato-dextrose agar. These results suggest that to assay the molds in flour it is necessary to use a medium on which the majority of the molds present will grow better than the other organisms present in the flour. The malt-salt agar seems to fill this requirement for the more than 60 flour streams we have sampled. This does not necessarily mean that it will be the best medium for assaying molds in all flour streams in all mills at all times. A slightly larger number of mold species have been cultured from certain flour streams on Smith-Humfeld agar than on malt-salt agar, but the malt-salt medium has consistently yielded a greater total number of mold colonies.

Consistency of mold count among replicate plates, or between assays of the same flour sample on successive days is not necessarily indicative of the suitability of the medium. The same sample of first

break flour was assayed each day for 10 successive days on Smith-Humfeld agar. Each assay consisted of four replicate plates. In most of these assays the flour was diluted 1:100, but some dilutions of 1:25 and 1:50 were also used, to permit a comparison of dilutions. An average of 550 molds per gram was obtained from the total of 40 plates. Five of the four-plate tests averaged between 550 and 575 molds per gram of flour. The number of molds on any one plate fell mostly within $\pm 10\%$ of the general average, and usually the variation was less than $\pm 5\%$. This was considered very satisfactory replicability. However, the same flour sample cultured by the same method on malt-salt agar yielded an average of approximately 1800 molds per gram.

Comparison of Different Methods of Sampling. Three methods of sampling were tested:

1. That described in detail later in the paper.

2. The flour sample to be assayed was diluted as in Method 1, and 1-ml aliquots of the suspended flour were flooded on the surface of a hardened agar medium. Best results with this method were obtained when the medium was prepared and poured into plates at least a week in advance of use. In that time the medium lost enough water to take up the 1 ml of liquid flooded over it within a few minutes.

3. A dry sampling method. For this, a small metal cup was welded to a wire inoculating needle. The cup was sterilized by flaming, cooled, and filled by pushing it through the flour. Tapping the wire lightly, with the cup inclined at a slight angle, served to remove excess flour from the surface of the cup and insure that it was level full. In numerous tests, the amount of flour delivered by this cup varied from 9.5 to 10.5 mg.

Enough sterile, dry quartz sand was poured into a sterile Petri dish to cover the bottom sparsely. A 10-mg cup of flour was added, and the dish shaken until the sand had distributed the flour particles evenly over the bottom of the dish. The cover was then removed from this dish, and from a Petri dish containing the agar medium. The bottom Petri dish containing the agar medium was inverted over that containing the sand and flour. Two rapid up-and-down motions served to bring most of the sand and flour against the agar, to which it adhered. The two dishes were then inverted, that containing the flour now being uppermost. The upper dish was tapped sharply, causing the flour adhering to it to fall to the agar. The culture dish was then covered and the process repeated.

Typical results of assaying two flour samples by these three methods are given in Table II. The same batch of malt-salt agar was used in all cases.

The three methods were compared extensively enough, with several

TABLE II

EFFECT OF METHOD OF SAMPLING ON THE NUMBER OF MOLD COLONIES OBTAINED FROM TWO FLOUR STREAMS

Method of sampling	Mold colonies per gram of flour	
	Sample 1	Sample 2
Liquid dilution		
Suspended in medium	1860	5920
On surface of medium	1780	3020
Dry, on surface	1080	4300

flour samples and different media, to demonstrate rather conclusively that a higher and more consistent mold count could be obtained by suspending a liquid dilution in malt-salt medium. If media wer used in which bacteria grew moderately well, the dry method yielded higher and more consistent mold counts than either of the other methods.

Comparison of Different Methods of Determining Number of Mold Colonies on Culture Plates. In the methods described in the literature, the number of mold colonies that grew in the culture dishes apparently was determined by visual examination of the dishes without magnification of any kind. In the present tests the mold colonies obtained from more than 10 different flour samples cultured on several different media with the three different methods of dilution were counted with the naked eye and with a binocular microscope. The magnification was approximately ×10. Typical results of the two methods of examining plates are given in Table III.

TABLE III

MOLD COUNT AS DETERMINED BY EXAMINING PLATES WITH THE NAKED EYE AND WITH THE AID OF A BINOCULAR MICROSCOPE [1]

Flour stream	Molds per gram [1]— examined by		Percentage increase [2]
	Naked eye	Binocular	
First break	1700	1800	6
First clear	2140	3720	74
Fine-1-tail	3400	5220	54
Suction	4240	5860	30
Coarse-2-tail	4125	7350	78

[1] Each figure is an average of five plates.
[2] Approximate percentage increase of binocular count over naked eye count.

The difference between the two methods of counting was less on those flour streams that contained a small number of colonies of a relatively few species of molds. Plates containing a total of 50 to 100 colonies of several different species of molds could be counted ac-

curately only with some sort of magnification. Many of the colonies of various molds grow slowly when submerged in the agar, and are invisible to the naked eye even when the cultures are 6 or 7 days old. At this age, some of the faster-growing surface colonies of *Aspergillus glaucus* and *A. flavus* began to obscure the small, submerged colonies, and the total as counted by either method began to decrease. There was a high agreement among replicate plates of a given flour sample with each method of counting, but the fact that more colonies were found when a binocular was used, in addition to the previously indicated fact that the composition of the medium affects the total count, shows that consistency alone was not a good criterion of accuracy.

Extreme Variation in Mold Count When Using Different Media and Different Methods of Examining Plates. One sample of flour cultured in the penicillin assay medium and read with the naked eye yielded 160 molds per gram; when cultured by the same method in malt-salt medium and read with the aid of a binocular a count of 1860 molds per gram was obtained. A second flour similarly assayed yielded 960 molds per gram when cultured in the penicillin assay medium and read with the naked eye, and 5920 per gram in malt-salt medium, read with a binocular.

Detailed Description of the Present Method

In view of the fact that several modifications of the methods described in the literature have been devised, it seems desirable to summarize the method of mold assay used in our laboratory.

REAGENTS:

Sodium chloride, C. P.
Malt extract, various commercial brands are suitable.
Agar-agar, crude shredded is suitable, but better grades may be used if desired.
Distilled water
Quartz sand

APPARATUS:

For each flour sample to be assayed the following are required:
Five 90-mm Petri dishes
Two 3-oz screw capped medicine bottles.
Two test tubes approximately 10 mm diameter and 6 cm long, calibrated to deliver 5 ml.
One 1-ml pipette. These are made from pyrex tubing approximately 4 mm inside diameter, drawn out at one end to an opening of 1.5 mm and calibrated to deliver 1 ml.
One rubber dropping bottle bulb to use with the pipette.
Torsion balance sensitive to 0.01 g.

PROCEDURE:

Preparation of medium. The medium is prepared in lots of 500 ml, sufficient to assay five flour samples. Boil 10 g of agar and 10 g malt extract in 350 ml of distilled water until agar is melted. Make up to 350 ml with distilled water. Dissolve 37 g sodium chloride in 150 ml distilled water. Place agar solution in a 16-oz screw-capped medicine

bottle, the sodium chloride solution in an 8-oz medicine bottle. Autoclave 20 minutes at 15 lb pressure. Add the sodium chloride solution to the agar and cool to 45°C before pouring. In practice, enough bottles of agar and sodium chloride solution are made up to last several weeks; the agar is melted in the autoclave as needed and the cold sterile solution of sodium chloride added to it.

Preparation of the flour. Weigh out 5 g of flour on a piece of sterile paper on the pan of a torsion balance sensitive to 0.01 g. Place in a 3-oz screw-capped medicine bottle containing 10 g sterile quartz sand. Using a large calibrated test tube, add 47 ml of sterile 0.5% solution of sodium chloride. Forty-seven milliliters are used because it has been found that 5 g of flour suspended in 47 ml of water make a total volume of 50 ml. Place screw cap on the bottle and shake bottle vigorously for 2 minutes. While the liquid is still agitated, and before any of the flour has settled, measure 5 ml into a second 3-oz bottle. This is done by means of a small test tube calibrated to deliver 5 ml. Add 45 ml of sterile 0.5% sodium chloride solution by means of a test tube calibrated to deliver 45 ml. Shake the bottle. While the liquid is still turbulent, remove 1 ml of the suspension by means of the pipette equipped with a rubber bulb, and immediately dispense into a sterile Petri dish. Shake the bottle again and dispense another 1-ml aliquot into another Petri dish. Usually five replicate plates are made of each sample. Cool agar to 45°C. Pour the cooled agar into the dishes, swirl each dish gently to suspend the flour uniformly, and place on a level table. When agar is hard, stack the plates on a shelf and allow to incubate for 5 to 7 days at room temperature (20°–25°C).

The calibrated test tubes and the improvised 1-ml pipette sacrifice a small amount of accuracy in measurement. This sacrifice is more than compensated for by increased speed of sampling, and in time saved in washing glassware. Five flours, with five replicate plates of each, can be sampled in two hours, including the washing of glassware. In our experience these measuring devices have proved equally as accurate as calibrated pipettes, so far as final mold count is concerned, as well as economical of time and labor.

Counting the colonies on the plates. Draw six or seven parallel lines on the bottom of each Petri dish with a wax pencil. Remove the cover and place the plate on the stage of a binocular microscope equipped with a mirror and an aperture in the stage through which the light can be directed. A desk lamp furnishes satisfactory illumination. With a magnification of x10 to x25, colonies throughout the entire depth of agar can be seen. The lines on the bottom of the plate serve as guides in moving the plate back and forth.

Use of controls. Samples of sterile flour are assayed from time to time as a check on the number of mold colonies that may enter from the air or other sources. The flour is sterilized by heating it slowly to 180°C in a gas oven, stirring it occasionally to prevent caking, after which it is left at 180°C for several hours. This sterile flour is assayed in the same way as are other flour samples. Replicate dilutions of 1:100 of this sterile flour seldom average more than 0.5 colony per plate.

Discussion

The present studies indicate that the number of mold colonies obtained from any given flour sample is influenced by the method of making the dilutions, the medium on which the flour is cultured, and the method of reading colonies in the culture dishes. While the malt-salt medium was superior to the other media tested, this does not imply that it will be superior in all cases. If flour streams whose mold

flora is unknown are to be extensively sampled, a number of different media probably should be tested.

The assumption is made that the approximately 4-oz sample taken from each stream is representative of that stream. Results of the dry assay method suggest that the molds are uniformly distributed in the flour. The mold count of individual plates among five replicates of a given flour sample by this method seldom varied more than ±5% from the average of the five. This relatively close agreement between replicate 10-mg aliquots of the same flour sample has been the rule, not the exception. This was true not only of the total number of mold colonies, but also of the relative numbers of each of the principal kinds of molds encountered in each sample. Evidently the molds were distributed with surprising uniformity in the samples taken from different streams. Further information on this will be presented in a later paper.

One advantage of examining the culture plates with a binocular microscope is that it permits one familiar with the molds to identify most of them at the time of counting. In some flour streams more than 10 species of molds, belonging to more than five genera, have been encountered, so that merely counting the total number of colonies does not furnish a complete picture of the nature of contamination.

None of the methods of mold assay now available distinguish between mold spores and fragments of mycelium. Nor do they permit one to estimate the number of nonviable spores that may be present. These deficiencies in the method do not seem important, but nevertheless they must be kept in mind. Information on these factors would be desirable in any interpretation of the role and significance of molds in flour.

Summary

Malt-salt medium was superior to the other five media tested, for determining the number of molds in flour. No medium was found on which both molds and bacteria could be assayed together.

The use of a binocular microscope increases the number of mold colonies found on agar plates when compared with the number found without the use of magnification. This is especially true when numerous colonies of several different species or genera of molds are present.

A higher mold count is obtained from a given sample of flour, using malt-salt medium, if the flour is suspended in sterile saline solution and this suspended in the agar, than if it is similarly suspended and cultured on the surface of the agar, or if it is spread dry on the surface of the agar. With other media, a higher count may be obtained by using the dry method.

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RELATIONSHIP OF ZEIN TO THE TOTAL PROTEIN IN CORN [1]

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The nutritional quality of corn germ protein has been recognized as approximating that of animal proteins, both from feeding experiments and comparative amino acid analyses (Block and Bolling, 1944; Mitchell and Beadles, 1944). Osborne and Mendel (1914, 1914a) demonstrated that the alcohol-soluble protein of corn, or zein, was nutritionally incomplete since animals rapidly lost weight on a diet containing zein as the only source of protein. Normal growth was restored by the addition of lysine and tryptophane to the diet. Zein was also demonstrated to be absent from corn germ although it constitutes the major protein of the endosperm. The rest of the endosperm protein, largely glutelin, was shown to contain the amino acids which zein lacks.

The available information regarding the distribution of lysine and tryptophane in different corn proteins is summarized in Table I. Within the limitations of present knowledge it would appear that the

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TABLE I

LYSINE AND TRYPTOPHANE CONTENTS OF VARIOUS CORN PROTEINS

Protein	Lysine	Tryptophane	Reference
	%	%	
Germ protein	5.8	1.3	(Block and Bolling, 1944)
Endosperm protein	1.1	0.6	(Block and Bolling, 1944)
Zein	0.0	0.0	(Osborne and Mendel, 1914)
		0.0	(May and Rose, 1922)
Glutelin	2.93	Strong positive color test	(Osborne and Mendel, 1914)
α-Glutelin	6.71	—	(Jones and Csonka, 1928)
Gluten	—	1.08	(May and Rose, 1922)

determination of zein content in a series of corn samples might provide an index of their relative feeding quality, on the assumption that the higher the zein content of corn protein the lower its feeding value. This analysis is much more easily carried out than are amino acid assays, and, in fact, consists in estimating the amount of protein with zero lysine and tryptophane content rather than directly determining these amino acids in the mixed proteins of corn.

The zein content of corn protein varies widely, values all the way from 28% to 60% having been reported (Showalter and Carr, 1922). The variation in values is due to several factors, among which are:

(*1*) *Method of Analysis.* The concentration of aqueous alcohol used by different investigators to extract the zein has varied from 70 to 95%. Recently it has been shown (Nagy, Weidlein, and Hixon, 1941) that addition of 0.5% sodium acetate as a buffer improves extraction of zein by 85% ethanol. These authors point out that the separation of different corn proteins by solvent extraction methods has proved far from satisfactory as a quantitative procedure, since large variations in the apparent percentages of the different fractions are caused by slight alterations in the procedure (fineness of grinding, length and temperature of extraction, presence of salts, etc.). Nevertheless they consider the alcohol-soluble fraction to be the most well-defined of these and least affected by slight variations in experimental techniques.

(*2*) *Maturity of Corn.* Zeleny (1935) found that zein was nearly absent in very immature corn but was synthesized rapidly as the corn approached maturity. The rapid increase in the ratio of zein to total nitrogen was almost exactly paralleled by the decrease in water-soluble nonprotein nitrogen.

(*3*) *Protein Content of the Corn.* Showalter and Carr (1922) were the first to point out that the proportion of zein to total protein is greater when the total nitrogen content of the corn is higher. An increase in zein was accompanied by a corresponding decrease in the

glutelin (dilute alkali-soluble) fraction. Using 90% alcohol as the extracting agent, they reported 8.1% zein in corn containing 15.7% protein and 2.2% zein in corn with 8.0% protein.

The present paper reports a more extensive study of the quantitative relationship between zein and total protein in whole corn and in corn endosperm.

Materials and Methods

The corn samples represented a varied assortment of inbred lines, single crosses, commercial hybrids; white and yellow corn; floury, dent, and waxy; high- and low-oil. The sweet corns included several waxy-sweet types; pseudo-starchy as well as extremely sweet samples; varieties such as Golden Bantam, Country Gentleman, and Evergreen; early and late maturing.

For alcohol-soluble protein, either the AOAC method (Association of Official Agricultural Chemists, 1940) or the following modification was used: Two grams of ground sample was weighed into a 250-ml centrifuge bottle, 50 ml of 70% ethanol added, and the stoppered bottle rotated continuously for 24 hours at room temperature. The contents were then centrifuged and 20-ml aliquots of the supernatant fluid taken for nitrogen analysis using the Kjeldahl method. On several samples of corn meal, 70% ethanol was found to extract the same amount of protein as did 85% ethanol buffered with 0.5% sodium acetate.

For separation of the endosperm, corn kernels covered with water were placed in a 50°C oven for 2 to 3 hours, after which the pericarp could readily be peeled off and the germ removed with a scalpel. After drying, the endosperm was ground to 40 mesh in a small Wiley mill. Total protein was determined on half-gram samples. All data were calculated to a moisture-free basis.

Results

The senior author illustrated that a highly significant correlation exists between zein and total protein content. Eighteen different corn samples covering the range from 6.3 to 19.7% protein were used. When 70% alcohol-soluble protein (Y) is plotted against total protein (X) the points fall along the regression line shown in Figure 1.

Data for 36 samples of endosperm from flour and dent corns are plotted in a similar manner in Figure 2 (solid line with circles) bearing out the linear relationship found for whole corn.

The amount of alcohol-soluble protein in sweet corn endosperm was found to be less than that in dent corn, averaging 1% less in the

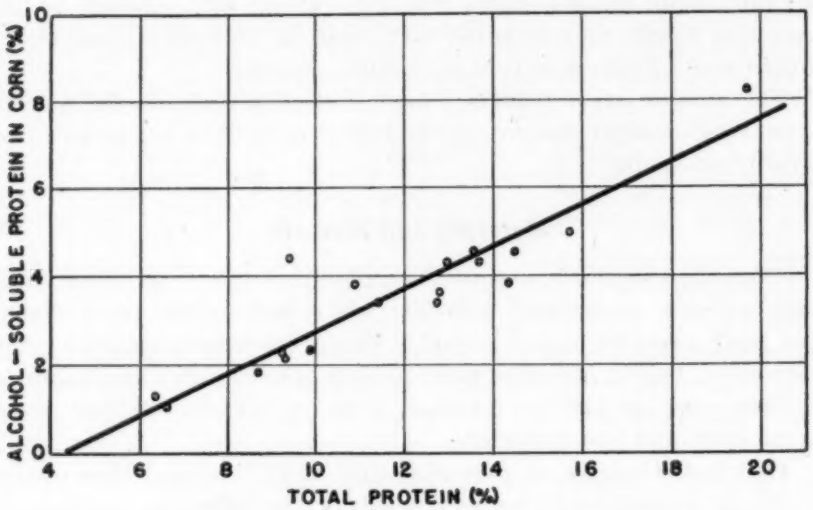

Fig. 1. Relationship between alcohol-soluble and total protein in dent corn.
$r = 0.923^{**}$ $Y = 0.478X - 2.0$

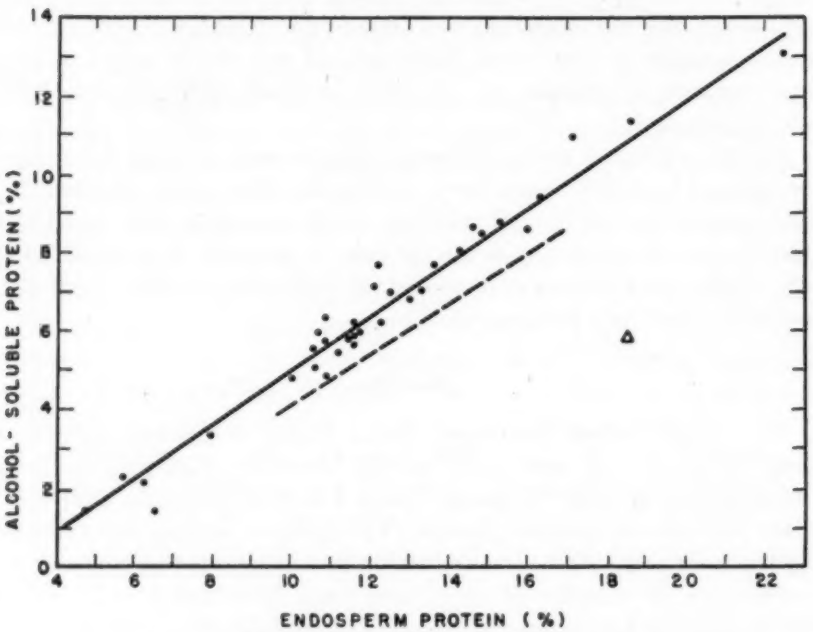

Fig. 2. Relationship between alcohol-soluble and total protein in corn endosperm.

Dent corn ——— Sweet corn - - - - - Brittle corn △

$\begin{cases} r = 0.984^{**} \\ Y = 0.692X - 1.87 \end{cases}$ $\begin{cases} r = 0.778^{**} \\ Y = 0.657X - 2.44 \end{cases}$

range measured (dotted line, Fig. 2). Thus at the 13% protein level, dent had 7.1% zein while sweet had 6.1%. Samples of sweet corn with extremely high or low protein content were not available and the correlation coefficient for the 30 samples analyzed, although highly significant, was lower than for the dent corns. This is not surprising in view of the variation in composition of corn classified as sweet. For example, the samples used varied from 14 to 42% in the amount of water-soluble constituents.

A third type of corn, known as "brittle," was also investigated (Mangelsdorf, 1926). It resembles sweet corn in appearance but is much more shriveled. The endosperm of one sample of brittle corn was found to contain 6.07% alcohol-soluble protein and 18.7% total protein, placing it far to the right of the lines for dent or sweet corn (Fig. 2).

Discussion

Prediction of nutritional values from analytical data is still in an uncertain state of development (Crampton, 1945). Nevertheless it seems logical that the feeding quality of corn protein would be inversely related to its zein content because of the latter's deficiency in the nutritionally essential amino acids, lysine and tryptophane. The value of the data presented in this paper lies in their use for detecting samples which depart widely from the average, in the direction of low zein content. Such samples could then be used in a breeding program with the hope of developing a high-protein, low-zein type of corn.

Mangelsdorf (1926) advanced the hypothesis that the maximum development of corn endosperm is reached in the normal starchy condition, and that the various endosperm types follow the same general growth pattern but may reach quite different end points when growth ceases. Using the weight of mature starchy kernels as a criterion he found that sweet corn reached only 88.5% and brittle corn only 62.5% of equivalent development; in other words, their seed weight when growth ceased corresponded to that of ordinary corn at earlier immature stages. This analogy may be extended to carbohydrate reserves. In dent corn the bulk of the carbohydrates is in the form of starch stored in organized granules. Sweet corn exhibits a less complete synthesis and a considerable part of the carbohydrate fraction is stored as water-soluble dextrins. Brittle corn is typified by an even less complete synthesis, the starch content being low and most of the soluble carbohydrates remaining in the sugar stage.

There are similar indications of incomplete development in the protein of these types of corn. On the basis of Zeleny's (1935) ob-

servation that the zein:total-protein ratio increases with advancing maturity, both brittle and sweet corn can be said to have zein:total-protein ratios corresponding to immature dent corn of equal protein content. In each case it appears that protein synthesis follows the same general sequence but that different end points are reached at maturity. For example, if a comparison is made of endosperm tissue containing 6% zein, the graphs show that this constitutes for dent corn 52.7%, for sweet corn 46.8%, and for brittle corn 32.5% of the total protein. The fact that the slopes of the regression lines in Figure 2 do not differ significantly suggests that synthesis follows the same general pattern in dent and sweet corn. This analogy is suggestive but should not be considered as an established fact.

Summary

Alcohol-soluble protein (zein) was determined on samples of corn and of corn endosperm having wide variation in protein content. The data indicate that there is a linear relationship between the content of alcohol-soluble protein and total protein.

Sweet corn endosperm contained an average of 1% less zein than dent corn of equal protein content. "Brittle" endosperm was found to be exceptional in its low zein:total-protein ratio. Apparently the protein as well as the carbohydrate in these types of corn differs from ordinary dent types in the proportion of its constituents.

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BOOK REVIEWS

Frontiers in Chemistry. IV. Major Instruments and Their Applications to Chemistry. Edited by R. E. Burk and Oliver Grummitt. viii and 151 pp. Interscience Publishers, Inc., New York, N.Y. 1945. Price $3.50.

This fourth volume of the Western Reserve University Frontiers in Chemistry is intended to tell the chemist what information may be obtained from the application of six different types of physical instruments to problems in various fields of chemistry.

Chapter I, "Electron diffraction and the examination of surfaces," by Lester H. Germer, is a reprint of an article by the same author in Jerome Alexander's "Colloid Chemistry," Volume V, 1944. It describes the use of electron diffraction cameras, or of slightly modified electron microscopes, for the study of the structure of surface films. These measurements give data analogous to X-ray diffraction patterns, but their limited penetrating power results in the pattern being produced only by the surface layer. Extremely minute samples are therefore adequate for an analysis.

Chapter II, "The electron microscope and its applications," by L. Marton, summarizes the theory of the increased resolving power and the construction of the electron microscope and gives pictures of some details of the author's machine. Applications to colloids, plastics, rubber, soaps, certain chemical reactions, metal surfaces, and biological material are described in sufficient detail to show the possibilities and limitations of the apparatus in various fields.

Chapter III, "X-ray diffraction and its applications," by Maurice L. Huggins, covers its field in more detail than the other chapters. The nature of X-rays, their production, and the systems used for taking the different types of photographs are described. The use of diffraction photographs for determination of the structure of known compounds and their use in identifying substances is described with many examples and crystal structure diagrams of both inorganic and organic substances.

Chapter IV, "Chemical spectroscopy," by Wallace R. Brode, briefly discusses the nature of light and the units used in measuring its wave length. The emission of spectral lines is illustrated by the energy level diagram of sodium atoms. The use of tables and charts for line identification is explained with examples, and a short description of the properties of the photographic plate is followed by a discussion of qualitative and quantitative analysis for metallic elements by emission spectroscopy.

Chapter V, "Application of absorption spectra to chemical problems," also by Brode, starts with the systems of units both for absorption coefficients and for wave length or frequency that are used in absorption spectroscopy. A particularly useful diagram is included which shows the same data plotted as percent transmission, extinction, (log I_0/I), and as log extinction. The connection between chemical structures and the absorption of light is clearly presented with absorption curves for various chromophores. Effects of conjugation and interference of various groups in organic molecules are illustrated by curves. The resolution of an absorption curve into its component bands is illustrated by $KMnO_4$ and by several organic compounds. This chapter is designed primarily to indicate how the organic chemist may use absorption spectra as an aid in elucidating the structure of a substance, since the purely analytical uses are already widely appreciated.

Chapter VI, "The infrared spectrometer and its application," by R. Bowling Barnes, gives a short history of infrared spectroscopy, describes the relation of infrared to other parts of the electromagnetic spectrum, and shows how infrared absorption spectra of organic molecules are related to the atomic groups present. Descriptions both of a large research model and of a smaller infrared spectrophotometer are given together with comments on the experimental difficulties involved in the design of such instruments. Typical applications to identification of organic molecules, determination of structure, quantitative analysis of several components of mixtures, measurement of reaction rates, and process control are clearly described as being an extension of the more familiar spectroscopic procedures to the infrared region.

Each chapter is contributed by a recognized authority in the special fields, but the book as a whole is so short that none of the chapters can be considered adequate to give a working knowledge of any of the techniques discussed. However, its purpose, to give the average chemist some information as to how the various instruments can be applied to different purposes, is well achieved.

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Organic Preparations. By Conrad Weygand. 534 pp. Interscience Publishers, Inc., New York, N. Y. 1945. Price $6.00.

This book is a translation of Part II, "Reaktionen," of Conrad Weygand's *Organisch-chemische Experimentierkunst*, published in Leipzig in 1938. Parts I and III of the German original, which deal with laboratory equipment and the chemical and physical analyses of organic compounds, have been omitted in the translation. The general plan and material in Part II of the German book have been followed quite closely. There is one major difference. The original contains numerous literal quotations of preparations published in *Organic Syntheses;* these have been replaced in the translation by references to *Organic Syntheses* and wherever possible references to single volumes of *Organic Syntheses* have been replaced by references to the Collective Volumes I or II.

The material is arranged by a new system based upon the formation of various linkages of the carbon atom and their fission. The method of organization may best be indicated by the chapter headings:

(1) Formation of Carbon-Hydrogen Bonds
(2) Formation of Carbon-Halogen Bonds
(3) Formation of Carbon-Oxygen Bonds
(4) Cleavage of Carbon-Oxygen Bonds
(5) Formation of Organic Derivatives of Trivalent Nitrogen
(6) Cleavage of Carbon-Nitrogen Bonds
(7) Formation of Carbon-Pentavalent Nitrogen Linkages
(8) Carbon-Divalent Sulfur Bonds
(9) Carbon-Hexavalent Sulfur Bonds
(10) Unsaturated Carbon Bonds
(11) Formation of Carbon-Carbon Bonds
(12) Fission of Carbon-Carbon Bonds
(13) Rearrangements of Carbon Compounds with the Exception of Steric Rearrangements.

Numerous methods for carrying out the transformations are discussed briefly and each type of reaction is characterized and its usefulness illustrated by specific laboratory procedures. This enables one to determine the applicability of a laboratory process and to select the best method for the solution of some particular problem. Many references to the literature make it possible to find further information concerning each method.

This translation makes available the most valuable section of Conrad Weygand's excellent German text. It fills the need for a working laboratory manual which is more advanced than the usual laboratory text books and which is up to date and gives more recent information concerning organic preparatory methods than is found in Houben-Weyl's classic handbook, *Die Methoden der Organischen Chemie.*

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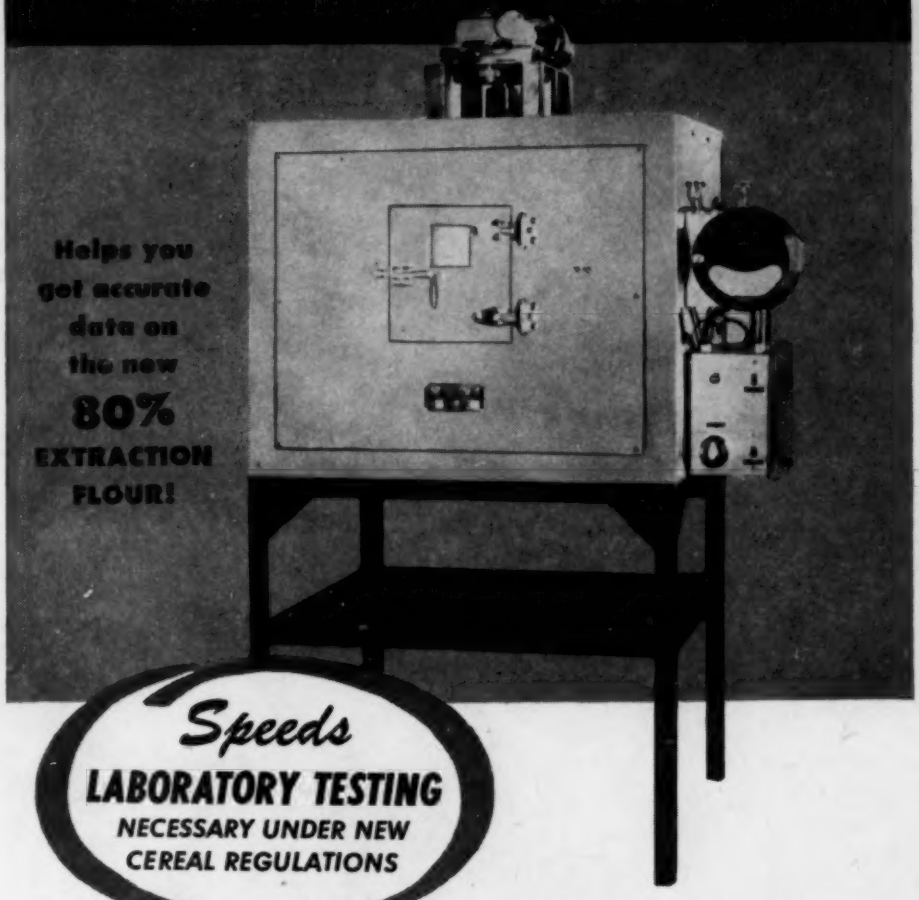

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